# PARASITISM OF NEZARA VIRIDULA (HETEROPTERA: PENTATOMIDAE) IN SOYBEAN AND OTHER HOST PLANT COMMUNITIES

By

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This work is dedicated to my friend Dr. Mario O. Vasconcelos who showed me the importance of a professional career and how it should be pursed.

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Ву

Luiz Antonio B. Salles

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Chairman: Dr. Jerry. L. Stimac

Major Department: Entomology and Nematology

Laboratory and field studies were conducted to determine parasitism of  $\underline{\text{Nezara}}$   $\underline{\text{viridula}}$  in nine host plant communities in Alachua County, Florida. The influence of parasitism on  $\underline{\text{N}}$ .  $\underline{\text{viridula}}$  performance in soybean is described quantitatively.

Rearing methods for <u>N</u>. <u>viridula</u> and <u>Trichopoda</u>

<u>pennipes</u> were developed and parasitization of <u>N</u>. <u>viridula</u>

adults by <u>T</u>. <u>pennipes</u> and eggs by <u>Trissolcus</u> <u>basalis</u> was

characterized.

In all host plant communities studied, during 1986 and 1987, N. viridula adults were consistently parasitized by T. pennipes, but nymphs (IV and V instars) were only occasionally parasitized. Eggs were parasitized by T. basalis. Both parasite species were present in all host plant communities sampled.

The  $\underline{N}$ . viridula population did not reach threshold levels during both years in soybean and levels of parasitization were lower in soybean than in other host plant communities studied.

<u>Solenopsis</u> invicta was the most common and important species of ant preying on  $\underline{N}$ . <u>viridula</u> egg masses in soybeans. An average of 68.5% of egg masses were preyed upon during 48 hours of exposure in soybean.

<u>Trichopoda pennipes</u> parasitization had a strong negative effect on  $\underline{N}$ .  $\underline{viridula}$  longevity, fecundity and feeding. In cages with parasitized  $\underline{N}$ .  $\underline{viridula}$  adults, 92.6% of seeds were normal compared with 78.2% for cages with unparasitized stink bugs.

 $\frac{\text{Trichopoda pennipes}}{\text{viridula}} \text{ adult body surface. There was no strong}$  relationship between abundance of tachinid eggs on host body and level of parasitization for either sex of the host.

## CHAPTER I

Food is a basic human need. Providing sufficient food of adequate nutritional quality for everyone is a priority objective for each of us that works on an arm of the hundreds in the food production system. Per capita food production is rising in some regions and falling in others. World agriculture is in a state of disarray (Brown 1987), due in part to production losses from pest organisms. The prevention of economic losses caused by pests was and will be a real challenge for human beings throughout existence on this planet.

Insects, weeds, and diseases became pests with the advent of modern agriculture and began interfering with human interests. Pest status, is relatively new, considering chronological time in the existence, for example, of insects on earth. When agriculture began, the world population probably did not exceed 15 million, no more than live in Greater London or Mexico City today. Inhabitants of the first cities were fed with grain surpluses produced in the immediate surrounding countryside. With the industrial revolution this changed. Urbanization became a reality and is still the dominant demographic trend of the late twentieth century. The

number of people living in cities increased from 600 million in 1950 to over 2 billion in 1986 (Brown and Jacobson 1987).

Cities are a relatively recent innovation, lagging by several millennia the emergence of agriculture some 12000 years ago. If urban growth continues at such rates, more than half of humanity will reside in urban areas shortly after the turn of the century. Therefore, farmers should maximize production in rural areas in order to be able to feed themselves and half of the world population. Although the pursuit of productivity (yield) has been central to agriculture since farmers first selected the wild grasses ancestral to crops, today the emphasis is on efficiency of agricultural production rather than only on higher yields.

Man continues to increase in numbers, currently the world's population is about 5.0 billion and by the year 2000 it will be around 6.5 billion (Brown and Postel 1987). It is obvious that our demand on the resources of the earth must be increased. The productivity from these resources must increase dramatically if the added population is to be supported, but ironically we are observing that as the human population continues to grow, the earth's biological systems are becoming less able to adequately support it. Few analysts expect a significant expansion of cultivated land. Just to maintain current consumption levels will require a 26% increase in the world's average grain yields,

and by 2020, feeding the projected population of 7.8 billion will require grain yields 56% higher than 1985 levels (Wolf 1986).

Direct and indirect consequences of pests are believed to be a major factor for the imbalance between production capability and the world demand for food and fiber. There is no question today that noxious insects are one of the major competitors of man who is trying to feed himself. Man cannot ignore the existence of insects (Barfield and Stimac 1980). Insect pests are considered among the major causes of yield reductions and pests have been considered responsible for 30-45% of crop losses throughout the world (Boardman 1986).

We must use the best technology available to reduce losses from insect competition as much as possible in order to produce more food, economically and healthily. Biological control of insect pests, the direct and indirect action of parasites, predators and entomopathogens on an insect pest population, offers one of the best solutions. Biological control reduces the actual trend on the use and burden of pesticides in the environment while deterring loss of food production efficiency.

Soybean, <u>Glycine max</u> (L) Merrill, a plant native to eastern Asia, was introduced into North America in the early 1800s (Kogan 1981). The presumptive center of origin of the cultivated soybean is located between parallels 40°

and 50° N in China. About 83% of the crop is now planted outside this area (Kogan and Turnipseed 1987). The cultivated soybean is typically a short season, temperate zone plant; 77% of the soybean area in China and 81% in the United States is found north of latitude 35° N, i.e. in the temperate zone. Worldwide, the area planted to soybean currently is about 52 million ha, in about 40 countries on all continents, and the area planted continues to expand (Kogan 1981). Soybean is presently the most important grain legume in the world and is used for food, medicine and oil. Much of the recent expansion has been to lower latitudes north and south of the equator. Kogan and Turnipseed (1987) called attention to potentially increasing vulnerability to insect pests under these new growth conditions. Subtropical and tropical fauna have a rich reservoir of potential soybean colonizers, and this fauna adapts rapidly to an introduced crop. The harmful effects of traditional soybean pests are expected to increase as larger areas are cultivated in the tropics. Biological control of new potential pests and those already adapted to soybean must be energetically encouraged if damage to soybean agroecosystems is to be avoided.

The purpose of the present work is to contribute to some degree towards efficient production of soybean through implementation of biological control for the southern green stink bug, Nezara viridula.

In Chapter II a general review of the literature is presented. Laboratory rearing methods for Nezara viridula and Trichopoda pennipes and the characterization of parasitism are presented in Chapter III. A study of the parasitism of  $\underline{N}$ . viridula in different host plant communities is presented in Chapter IV. In Chapter V  $\underline{N}$ . viridula population development and its parasitism in soybeans are described. In Chapter VI the effects of  $\underline{T}$ . Pennipes parasitization on  $\underline{N}$ . viridula longevity, fecundity and feeding are described. Trichopoda pennipes oviposition on  $\underline{N}$ . viridula adults is presented in Chapter VII. Chapter VIII contains the summary and conclusions from all studies.

#### CHAPTER II REVIEW OF THE LITERATURE

#### Introduction

The literature on soybean phytophagus pests and their host-parasitoid complex is vast, and the choice of examples is illustrative, not exhaustive, and is concerned mainly with Nezara viridula and its major species of natural enemies.

The contemporary species composition and structure of arthropod communities associated with a crop are the result of a highly dynamic adaptative process. Arthropod communities on soybean are less than 50 years old in about 80% of the area currently under cultivation to this crop (Kogan 1981, Kogan and Turnipseed 1987).

Kogan and Turnipseed (1987) presented a general picture of the soybean arthropod pests in various regions of the world: in the United States, they listed 453 phytophagus species of which 14 are major pests. They also reported total phytophagous species and major pests respectively for Brazil 150 (16), India 122 (14), Japan 241 (16), Korea 120 (14). As we can observe, the number of major insect pests in soybean worldwide is about the same magnitude. They also named the major pests in each these regions and concluded that by comparison with soybean arthropod communities in the Orient, those in North and South America

seem to be immature. Niches in North and South American soybean ecosystem are occupied by many polyphagous species and by oligophagous species that use soybean as a secondary host. Insect communities in the New World seem to have few well-adapted species that feed on soybean.

In their bibliographic review of N. viridula, DeWitt and Godfrey (1972) pointed out that the review was not limited to papers dealing with the southern green stink bug on soybeans, and they listed 629 references, covering a period prior to 1800s to the 1970s. Panizzi and Slansky (1985) presented a literature review of the phytophagous pentatomids associated with soybean in the Americas, and they listed 206 references.

In 1976, Turnipseed and Kogan (1976) published a review on soybean entomological literature and found 264 references. Interestingly, in 1986 they found nearly 5000 references from the same data base (Kogan and Turnipseed 1987). Obviously, in only 10 years the entomological research effort on soybeans had increased significantly throughout the world.

## Taxonomy and Geographical Distribution of Nezara viridula

Nezara viridula (Heteroptera:Pentatomidae) was first described by Linnaeus in 1758, under the scientific name Cimex viridula, based on specimens collected in India. The species is now placed in the genus Nezara Amyot and Serville 1843 (Pentatominae:Pentatomini), Freeman (1940).

Jones (1918) and Freeman (1940) gave a general morphological description of N. viridula life stages. Both Jones (1918) and Freeman (1940), as well as other sources, described N. viridula adults with a general green color; however, the southern green stink bug, N. viridula, is polymorphic both in the nymphal and adult stages. Kiritani (1970) stated that the variation in body color observed among nymphs of 4th and 5th instars is controlled to some degree by environmental conditions, e.g. temperature (Kariya 1961) and population density (Kiritani 1963a). By contrast, the color types of adults seem to be under genetic control. At least four fundamental color types can be distinguished without intermediate ones in N. viridula (Kiritani 1970). Yukawa and Kiritani (1965) studied N. viridula adult polymorphism in Japan, with local and foreign fauna. They presented several types: named smaragdula Fabricius (color pattern entirely green); torquata Fabricius (median and lateral lobes, anterior margin of pronotum yellow); viridula Linneaus (green spots on yellow ground color); aurantica Costa (entirely yellow, orange or pink); vicaria Walker (entirely brown); chlorocephala Westwood (entirely brown). DeWitt (1972) states that nine different morphs may exist throughout the world with the greatest expression of polymorphism present in south-eastern Asia. For this reason Yukawa and Kiritani (1965) suggested that this part of Asia be considered as

the original home of  $\underline{N}$ .  $\underline{viridula}$ . Yukawa and Kiritani (1965) emphasized that in reference to geographical variation of a species, it is very important to consider not only selection by environmental factors after establishment, but also the initial genetic composition of the imported population. The relative frequency of polymorphs among newly established populations of  $\underline{N}$ .  $\underline{viridula}$  is primarily determined by the initial genetic drift and natural selection. Hokkanen (1986) suggested that the native area of  $\underline{N}$ .  $\underline{viridula}$  is probably in the Ethiopian region rather than in the Indo-Malaysin region. His conclusion was based on the equal diversity of morphs found elsewhere.

Sailer (1981) sustained what Kiritani (1970) reported that only the green form of N. viridula occurred in North America, because to his knowledge no published evidence to the contrary existed in 1975. In 1975, Dr. Reece I. Sailer (personal communication) decided to utilize N. viridula for research on inbreeding expression and started culturing the species from wild green stock collected at Gainesville, Florida. In the third generation of one line (he used three lines) a quarter of the adults were not the expected green but were a yellow color which he designated as "gold." Dr. Richard Baranowski in 1978 sent to him a live gold male collected at Homestead, Florida. Phenotypically it was identical to Dr. Sailer's Gainesville golds, but

when mated to three different Gainesville gold females only green progeny were produced. This gave him evidence that a recessive allele, at each of two loci, controlled the expression of the gold phenotype. Dr. Sailer spent the last years of his life intensively and enthusiastically working on this fascinating problem, and he was convinced that he had answered the question of how the gold color of the southern green stink bug is determined.

Dwinell (1984) studied the cuticular hydrocarbons content from males and females of a gold phenotype and the common green phenotype of N. viridula. These extracts were analyzed using gas chromatography. No differences were detected between green and gold phenotypes. Sailer (1981) determined that such differences are due to genetic makeup in these insects, and genetic polymorphism (the various forms are attributable to genetic differences) should be, then, a preferred term for a broad classification of such phenonemon.

Despite the controversy of its origin, N. viridula undoubtedly was imported from its original home into various areas and currently is widely distributed throughout the world. DeWitt and Godfrey (1972) stated it is found in the Neartic, Neotropical, Australasian, Oriental, Ethiopian and Paleartic biogeographic regions or realms as preferred by Price (1984). A distribution map published by the Commonwealth Institute of Entomology was

reproduced by DeWitt and Godfrey (1972) and Todd and Herzog (1980). The southern green stink bug is one of the most widely distributed species of plant feeding insects, and Todd and Herzog (1980) stated that it is by far the most cosmopolitan of the pentatomids attacking soybean. As mentioned by DeWitt and Godfrey (1972), the first new world record of the species is from the West Indies, according to Fabricius in 1798, who called it <u>Cimex spirans</u>.

Jones (1918) has pointed out that in common with many other insect pests in the United States, N. viridula was introduced from the West Indies and currently is established over a large area south of a line extending from Texas in the west through southern Arkansas to Virginia in the east, reported from at least 11 continental states (AL, AR, FL, VA, GA, CA, LA, NM, MS, SC, TX), Turnipseed and Kogan (1983). Jones (1918) presented a map showing the distribution of the southern green stink bug at that time in the United States. This species appeared to be concentrated mostly in the states bordering the Gulf of Mexico. Recently, a map of N. viridula distribution in the United States was prepared by the USDA (1975). In Florida, it occurs throughout the state, and probably major populations are restricted to and/or concentrated in areas where suitable hosts occur rather than by any major physical barriers.

Nezara viridula is known by various common names: cotton green bug, green bug, green tomato bug, pumpkin bug, southern green plant-bug, and it was Jones (1918) that proposed the common name of the southern green plant-bug and the word "southern" was used to distinguish it from the closely related species N. hilaris Say. Drake (1920) coined the currently used common name, southern green stink-bug, which has been universally accepted and then approved by the Entomological Society of America as southern green stink bug.

## Life History and Behavior of Nezara viridula

The life history and behavior of the southern green stink bug have been studied by several workers in different parts of the world, but especially by Japanese researchers: Kariya (1961), Kiritani and Hokyo (1962), Kiritani (1963a, 1963b), Kiritani and Kimura (1965), Kiritani et al. (1966a, 1966b), Kiritani (1967), Kiritani et al. (1967), Kiritani (1969), Kiritani and Sasaba (1969), Kiritani (1970), Kiritani (1971), Hokyo and Kiritani (1963), Nakasuji et al. (1965). Most of these studies were developed, so intensively, probably because N. viridula is important as a pest of paddy rice and vegetables in southern Japan. Rice recently has been reintroduced into the Everglades agricultural area of southern Florida, after a 20 year absence. In 1985, rice was grown on about 4000 ha in this area. Although, many different insects can be

found in rice fields in the Everglades, stink bugs are currently considered the most important pests. In 1977, Genung et al. (1979) found five species of pentatomids, among them N. viridula, and Jones and Cherry (1986) found four species in the same geographical area and N. viridula represented only 1.5% of the stink bug population sampled during the 1983 and 1984 rice seasons.

In general, information on life cycle, generation time and behavior are much the same and variations are usually dependent on seasonal temperature at a given location.

In the United States, the classical works of Jones (1918) and Drake (1920) were the most complete. More recently, several researchers in the southern United States, especially in Georgia, have intensively studied N. viridula, Mitchell and Mau (1969), McLain (1981), Harris and Todd (1980a, 1980b, 1980c), Harris et al. (1982), Harris et al. (1984), Banerjee and Chatterjee (1985). These authors indicated that this insect, like most other pentatomids, overwinters in the adult stage, under litter, bark, and other objects which offer protection. Adults. under Florida and other southern states climatic conditions, have only a partial hibernation which R. I. Sailer (personal communication), preferred to refer to this as diapause instead of hibernation. Adults may remain and feed on plants throughout the entire winter (Drake 1920 and R. I. Sailer, personal communication). Insects that

are not developing may be said to be in developmental arrest or dormancy. Summer dormancy is aestivation and winter dormancy is hibernation. These states of dormancy may also be classified on the basis of the circumstances required for ending the dormancy. Quiescence is a dormancy development that is directly controlled by unfavorable conditions. Development stops when environmental conditions become unfavorable and resumes when they again become favorable. Diapause is a dormancy development that persists even when environmental conditions are such that they would ordinarily favor development. In diapause, development often stops before environmental conditions become unfavorable and its resumption depends on the ending of some internal physiological block. Distinguishing between quiescence and diapause is sometimes difficult as stated by Walker (1986). There are no winter broods and only adults are found during the winter. In the early spring, adults begin to leave their overwintering sites and start their search for food. Drake (1920) pointed out that mating begins almost immediately.

The female and male, when mating, remain attached to one another by the tips of their abdomens with their heads facing in opposite directions. Under natural conditions copulation is repeated a number of times, either for male or female, but this is not clear for N. viridula behavior under field conditions. Harris and Todd (1980b) reported

that in studies of caged insects, 45% of the time is spent in copulation. Laboratory studies of  $\underline{N}$ .  $\underline{viridula}$  reveal that the duration of copulation and number of copulations do not correlate with the number of fertilized eggs that the female lays and that males guard females via prolonged copulation to protect the precedence of their ejaculates (McLain 1981). Mitchell and Mau (1969) studied  $\underline{N}$ .  $\underline{viridula}$  mating behavior under laboratory conditions.

The only reference found that studied the ethology of the southern green stink bug in detail was developed by Lockwood and Story (1986b) on cowpea. The purpose of their study was to develop a behavioral catalogue for N. viridula and analyze the frequency and duration of behaviors in context of the biology and temporal/spatial ecology of the insect. No similar work was carried out in soybean until the present. Wait (1980), in Australia, studied over a short period of time (April 9 to 23) the basking behavior of N. viridula on soybean. He noted that upward movement of this insect commenced around 7:00 AM and peak numbers were recorded on the surface of the plants between then and 9:00 AM. From 9:00 to 12:00 there was a continuous decline in the number of bugs on the surface. Generally, only a few individulas were observed there after 11:00 AM. although a slight increase in surface numbers sometimes occurred during late afternoon from 4:00 until dusk. Both adults and nymphs displayed diurnal movement. He concluded

that numbers of bugs on the surface were best correlated with time of the day, temperature and humidity, but these factors accounted for only 32% of the variation in numbers of bugs. On days with significant cloud cover the morning basking period was extended and more insects could be found around midday.

Drake (1920) observed and described the process of egg-laying in the laboratory. Egg-laying, according to Drake (1920), under natural conditions in Gainesville begins about the middle of April. Eggs are deposited in a mass, or compact cluster, in which the individual eggs are arranged in very regular rows and firmly glued together. The egg mass is normally deposited upon the surface of the host plant and generally on the underside of the leaf. At time of deposition the eggs are light yellowish and shiny. Gradually, they become somewhat yellow opaque or cream colored, and later in the incubation period the eggs begin to turn pinkish, and a red crescent shaped spot appears on the operculum. These colors gradually grow deeper and more conspicuous until eclosion (Todd and Herzog 1980). Harris and Todd (1980c) observed that egg masses deposited on the same day did not always exhibit the same incubation period, and they attributed this to inherent differences among the egg masses. All individuals from the same egg mass hatch on the same day (Jones 1918, Drake 1920, Harris and Todd 1980c). Stink bug nymphs almost invariably pass through

five instars (Slater and Baranowski 1978) and the southern green stink bug is not an exception. During the first instar, nymphs cluster on or very near the egg shells. First instar nymphs do not feed. Kiritani (1969), Singh (1972) and Lockwood and Story (1986a) stated that N. viridula is gregarious in its first three instars. dispersing by the fourth instar. Nezara viridula has a complex populational profile; it is polyandrous, ploygynous, multivoltine and long-lived as an adult, thus producing overlapping generations (Harris and Todd 1980c). These factors complicate the study of its population dynamics, and difficulties arise in attempting to ascertain instar duration, which depends on prevailing photoperiod, temperature, and humidity, as well as on quality and quantity of available nutritional resources. At least ten studies have been conducted on the immature life-stage duration of N. viridula, performed under different conditions (temperatures, techniques, photoperiods, latitudes, etc.). These studies vary considerably in their results (Jones 1918, Drake 1920, Kiritani and Hokyo 1962, Corpuz 1969, Singh 1972, Velez 1974, Harris and Todd 1980a, 1980c, Correa 1985), but the range and mean duration of each immature stage among these studies were: egg, range of 4-6 (mean of 5); first instar 3-5 (3); second instar 3-12 (6); third instar 2-11 (6); fourth instar 3-10 (8); fifth instar 7-18 (11); egg to adult 22 - 40 (35) days.

## Host Plants and Economic Importance of Nezara viridula

The southern green stink bug is a phytophagous insect with a broad range of host plants, including cultivated and wild ones, field and vegetable crops. Nezara viridula feeds on both monocotyledons and dicotyledons. Among the former, the Gramminae are the most important, and within the dicotyledons about 29 plant families are used as food and/or injured (Leguminosae: 27; Cruciferae: 8; Solanaceae: 6 species) according to Todd and Herzog (1980), Jones and Cherry (1986), and Negron and Riley (1987).

Drake (1920), Kiritani (1971), Todd and Herzog (1980) presented a long list of plants that the southern green stink bug utilizes in nature. The most common economically important crops listed were raddish, mustard, collard, cauliflower, cabbage, okra, peas, tomato, potato, tobacco, brussels sprouts, cotton, eggplant, sunflower, sugarcane, corn, rice, pecan, lime, lemon, peaches, grapefruit, wheat, orange, berries, beans, and cowpeas. Link and Grazia (1987), as a result of their survey in southern Brazil, included other plant hosts on which N. viridula is considered to be a pest: alfalfa, rapseed, lentil, flax, green pepper and oats. The southern green stink bug is a polyphagous species in the strictest sense of the word.

Importance of its damage, certainly is directly related to several local factors of the crop situation,

including potential yield, marketability of product and supplies, need of the product (subsistence or exportation), producer objective, intended use of product. But, regardless of these considerations, the southern green stink bug is generally considered a severe pest to all kinds of beans, tomatoes, corn, peas, okra and soybeans. Numphs and adults cause irreversible direct damage to developing fruits and seeds (Panizzi and Slansky 1985), reducing yields, product appearance, nutritional qualities and some sort of indirect damage because products attacked are more susceptible to mold in storage.

Disease transmission and/or association with  $\underline{N}$ .  $\underline{viridula}$  feeding was mentioned by Daugherty (1967), Ragsdale (1977), Franca Neto and Henning (1984) for the fungus  $\underline{Nematospora}$   $\underline{coryli}$  Peglion causing deterioration of soybean seeds. However, Kilpatrick and Hartwing (1955) demonstrated that injury by the southern green stink bug was not necessary for invasion and infection of soybean seed by this organism.

The long list of food plants shows that the insect is a very general feeder or highly polyphagous (Todd and Herzog 1980), and can subsist upon a great variety of plants. However, it probably does not reproduce, at least in great numbers, on all of these plants and feeds only occasionally on a number of them. We should discriminate the plants on which it can complete its life cycle and

reproduce, and those on which adults utilize for subsistence. Certainly, this list of hosts could be greatly reduced.

In fact, the greatest economic importance of  $\underline{N}$ .  $\underline{viridula}$  is because it is the most important phytophagous pentatomid pest of soybean in the Americas (Panizzi and Slansky 1985). In the southeastern United States, it is listed among the major pests of soybean (Ducan and Walker 1968, Todd and Herzog 1980, Kogan 1981). Turnipseed and Kogan (1983) reported an economic loss of 13.4% throughout this soybean producing area. Probably for these reasons, it is the most studied species in the family Pentatomidae. Todd and Herzog (1980) cited as the first reference stating that  $\underline{N}$ .  $\underline{viridula}$  feeds on soybean was by  $\underline{N}$ . E. Hoffman in 1935.

## Natural Enemies of Nezara viridula

An assessment of the role of natural enemies of insect pests has been of major importance to entomologists since the inception of biological control. It is particularly significant in soybean entomology because of the abundance and great diversity of natural enemies in soybean agroecosystems (Pedigo et al. 1983).

Regulation of the southern green stink bug population is attributed to several biotic and abiotic factors.

Kiritani and Kimura (1965), from work done in Japan, suggests that mortality factors work in a stage-specific

way, i.e. parasites against eggs and last numphal stage and adults, weather factors against the first instar and predators against the second.

#### Predators and Diseases of Nezara viridula

The importance of predator complexes, or interacting groups of arthropod predator species that function as a unit in inflicting mortality, in pest populations has long been recognized (Elvin 1983). Predators are major causes of pest mortality in soybeans and researchers have recently attempted to quantify predation rates (Elvin 1983, Elvin et al. 1983, Grant and Shepard 1985). Herzog et al. (1985) suggested that predators which are generalists in feeding behavior, with relatively long generation times and low reproductivite capacity, act as buffers against pests which first colonize the crop. Mortality, therefore, is inflicted principally on eggs and early life stages of pests.

Pathogens have been incorporated into soybeans ecosystem through intentional manipulations. The use of industrial produced pathogens (Kogan and Turnipseed 1987) and Nomuraea rileyi remains the most important fungal disease of soybean lepidopterous pests. There are several other pathogens (e.g. Entomophora spp, and virus) reported on important soybean insect pests but their impact on pest populations is considered to be minimal. No mention of

successful control infection by pathogens of pentatomids in soybean was found.

#### Parasites of Nezara viridula

Jones (1988) provided an excellent world review of the parasites of N. viridula with a survey of published and unpublished information. He listed 46 species of parasites among two families of Diptera and five families of Hymenoptera. He also states that no hyperparasites are known from N. viridula parasites, but Exoristobia phillippinensis Ashmead is mentioned as a pupal hyperparasite of Trichopoda pennipes Fabr. (Davis 1964), and he stated that there are no known effective parasites of the nymphal stage.

Five species of Tachinidae (Trichopoda pennipes, T. pilipes (Fabricius), T. giacomelli (Blanchard),

Eutrichopodopsis nitens Blanchard, and Gymmosa rotundatum

L.) are known to attack adult N. viridula (Liljesthrom 1980, 1981, Correa 1984, McPherson et al. 1982, Todd and Lewis 1976) on a regular basis in geographically separated areas, respectively in the United States, the West Indies, Argentina, Brazil and Japan.

<u>Trissolcus basalis</u> (Wollaston) is the most widespread egg parasite and now occurrs with  $\underline{N}$ .  $\underline{viridula}$  in the New World (Brazil and Argentina), parts of Africa, southern Europe, Asia, Australia, New Zealand. Hokyo and Kiritani (1966a) and Nakasuji et al. (1966) reported two species of

scelionids, <u>Asolcus mitsukurii</u> Ashmead, and <u>Telenomus</u> nakagawai Watanabe as the principal egg parasites of the southern green stink bug in Japan.

Five families of Hymenoptera have been recorded emerging from eggs of  $\underline{N}$ .  $\underline{viridula}$  but, on a worldwide bases, the Scelionidae is by far the most important.

Buschman and Whitcomb (1980) collected N. viridula eggs, adults and nymphs around Gainesville, FL, during 1974-75, from several host plants and concluded that only Trichopoda pennipes and Trissolcus basalis were consistently present and they considered these two species the only two important N. viridula parasites in north Central Florida. Drake (1920), 60 years earlier, mentioned Trichopoda pennipes as the most important natural enemy of the southern green stink bug in this area, and he mentioned that, occasionally, the eggs are parasitized by "minute four-winged insects that are commonly known as chalcid flies, belonging to the superfamily Chalcidoidea."

Identification of this egg parasite was not very clear, but he mentioned it as belonging to the genus Trissolcus, probably T. euchisti Ashmead.

In South Carolina, Georgia and Louisiana, the most abundant species of parasites of N. viridula are also Trichopoda pennipes and Trissolcus basalis (Todd and Lewis 1976, McPherson et al. 1982, Harper et al. 1983).

Differences in natural enemy composition and density are often pronounced throughout the growing season at a location and between locations, and the meaning and significance of these differences impact strongly on control options of soybean insect pests and upon the overall design of pest management strategies used in soybean production.

Newsom et al. (1980) and Pedigo et al. (1983) stated that published studies dealing with parasites of soybean insect pests are relatively few compared to those of other natural enemies, and they believe that a possible reason for this is that parasites have a minor impact on soybean insect populations, but they call attention to the cryptic nature of many parasites, which probably has contributed greatly to the reason many important species go unnoticed. A complex of hymenopterous and dipterous parasites has been defined, and some attempts have been made to determine impact or overall effectiveness of these on soybean insect pests, and particularly for the southern green stink bug. Recently Jones (1988) reviewed the literature published on these parasites.

Trissolcus basalis (Hymenoptera:Scelionidae) was first described by Wollaston in 1858, from specimens collected on the Ilha da Madeira. Wilson (1961) and Loiacono (1980) listed the following synonymns for Trissolcus basalis (Wollaston): Telenomus basalis Wollaston, 1858; Telenomus

maderensis Wollaston, 1858; Telenomus megacephalus Ashmead, 1894; Telenomus piceipes Dodd, 1919; Liophanurus megacephalus Kieffer, 1926; Asolcus basalis (Wollaston) Nixon, 1935; Asolcus basalis (Wollaston) Delucchi, 1961. Wilson (1961) reported this species as Asolcus (Microphanurus) basalis (Wollaston) (=Telenomus megacephalus Ashmead). Kamal (1937) reported the species Microphanurus megacephalus (Ashmead) as an important egg parasite of N. viridula in cotton in Egypt.

Trissolcus basalis has been reported as a polyphagous parasite with a broad range of dispersion throughout the world. It has been reported in Europe, Asia, Africa, North and South America (Cumber 1949, Davis 1964, Hokyo and Kiritani 1965, Kamal 1937, Wilson 1961, Loiacomo 1980, Correa 1980, 1986).

Since N. viridula is a known immigrant to areas outside Africa and southern Asia, there have been several attempts to establish T. basalis into new areas invaded by the southern green stink bug, mainly in the Pacific Basin (Wilson 1961). The programs in Hawaii and Australia have been considered very successful, Messenger et al. (1971) and Caltagirone (1981) included the southern green stink bug as a landmark example in classical biological control, especially for the efforts and successes obtained with the introduction of T. basalis in Australia, New Zealand, Fiji, Hawaii, and various other islands in the Pacific. The

parasite species was introduced in Brazil and Argentina where it became very well established (Loiacono 1980, Crouzel and Saini 1983, Correa 1986).

The egg parasite, <u>Ooencyrtus submetallicus</u> (Howard), was imported in 1953 from the West Indies and released in central Florida. It has become established, but apparently provides little control (Buschman and Whitcomb 1980, Jones et al. 1983). According to Jones et al. (1983), an importation program was initiated in 1979 by the USDA Southern Field Crop Insect Management Laboratory to screen egg parasites of <u>N. viridula</u> for possible release and establishment, and several species have been received, and releases of an uniparental form of the scelionid, <u>Telenomus</u> sp. near <u>chloropus</u> Thomson (=<u>T. nakagawai</u> Watanabe) from Japan were started in Mississippi in 1981 and in Louisiana in 1982.

Insects of the same species from different geographical locations are often characterized by biological differences, and these differentiated populations are called races, strains or biotypes, and may be very important in biological control, especially in classical biological control programs (Messenger et al. 1971, Caltagirone 1985).

Several strains of  $\underline{T}$ . <u>basalis</u> have been studied and differences in their biology reported (Kamal 1937, Powell and Shepard 1982, Correa and Zamataro 1986). Wilson (1961) reported one of the most comprehensive studies on T.

basalis reproductive behavior, and Kamal (1937), Powell et al. (1981). Powell and Shepard (1982) and Orr et al. (1985) complemented his work by studying other aspects of its general biology. Wilson (1961) indicated that T. basalis is a solitary, arrhenotokus parasite, which completes development from egg to adult within the host egg. It is a multivoltine species, passing through a number of generations each year and development is correlated to temperature. At 27°C, for example, the males lived for 4 to 5 days and the females for 4 to 15 days. Sales (1985) confirmed that at 27°C and 65% RH, male T. basalis have a life span varying from 3 to 5 weeks while the females live for 4 to 15 weeks, and he also recorded that females are able to live as long as 10 months with a honey and water supply, under mentioned conditions. Wilson (1961) indicated that the number of offspring produced by individual females varied between 104 and 187. The females begin to oviposit on the day of emergence and, in the presence of ample hosts, deposit practically all their eggs during the first 4 or 5 days (Wilson 1961). Accurate details of T. basalis oviposition behavior is given by Wilson (1961) and he also pointed out some of the most peculiar characteristics for T. basalis: 1) marking of the host egg after parasitization; 2) female ability to discriminate between parasitized and unparasitized hosts and to exercise restraint in oviposition; 3) aggressiveness of the ovipositing female; 4) diurnal periodicity of adult emergence and emergence of males before females; 5) male aggression leading to possession of an egg mass by one male; 6) immediate fertilization of each female on emergence; 7) high mating capacity of the male, fertilizing of nearly all females by one male; 8) numerical preponderance of females.

Some of the most important aspects of phytophagus insects are the mechanisms used to find a host plant. Although many scientists throughout the world have studied various insect-plant interrelationships, we still only have a superficial knowledge of the mechanisms. Parasites are not an exception of the above rule. For both, parasites and phytophagous insects we have to recognize that only basic ideas, mechanisms, and processes for very few species are outlined in the evolutionary process of parasite-prey interaction. Sales (1985), Bin and Vinson (1985), and Bin et al. (1985) studied interspecific communication between T. basalis and N. viridula, indicating that physical and chemical stimuli are present, such as some specialized antennal structures of T. basalis and an egg recognition kairomone is present.

Jones (1988) stated that the only known insect parasites which attack adult  $\underline{\text{N}}$ .  $\underline{\text{viridula}}$  are the Tachinidae. However, Burks (1972), Bushcman and Whitcomb (1980) mentioned Hexaclidia hilaris Burks

(Hymenoptera: Encyrtidae) as a primary parasite of the adults. In Japan, N. viridula is parasitized by Gymnosoma rotundatum L., a widely distributed tachinid in the Old World and up to 5% parasitism was reported by Kiritani (1963b). Jones (1988) mentioned that there are no known records of tachinids regularly attacking N. viridula in Europe and Africa. In the Americas, where N. viridula is a known immigrant, it is heavily attacked by four related native species, each separated geographically: Trichopoda pennipes (F.) in the United States (Todd and Lewis 1976. Buschman and Whitcomb 1980, McPherson et al. 1982); T. pillepes (T. pennipes pilipes (F.)) in the West Indies, introduced into Hawaii and Australia (Arnaud 1978, Michael 1981, Shahjahan, 1968); Euthrichopodopsis nitens Blanchard in Brazil (Correa 1984) and T. giacomellii Blanchard in Argentina (Liljesthrom 1980).

Trichopoda pennipes was first described by Fabricius in 1794 as Musca pennipes, but later (1805) the genus was changed to Dictya. He also described (1805) Thereva hiertipes, Trichopoda pennipes, and Ocyptera ciliata which have all proved to be synonymous with T. pennipes.

Latreille (1825) erected the genus Trichopoda, into which the Dictya pennipes Fabricius was placed by Wiedemann (1830) and Robineau-Desvoidy (1830), as cited by Beard (1940).

Trichopoda pennipes has no popular name in common use, but it has been named the feather-legged fly, because of a prominent fringe of feather like setae on the outer side of the hind tibia (Bradley 1939, Todd and Lewis 1976).

Trichopoda pennipes, according to Arnaud (1978), parasitizes 32 species distributed in 5 families in the order Hemiptera (Heteroptera) in the North American region. Beard (1940) stated that, of the genus Trichopoda, which belongs to the fauna of the Americas, T. pennipes is the most widely distributed species, in both North and South America and among adjacent islands.

A detailed account of the biology and habits of the species is given by Drake (1920), Worthley (1924) and Beard (1940), especially the last two.

Dietrick and Van den Bosh (1957) described a rearing method for  $\underline{T}$ . pennipes and Michael (1981) called it a tricky parasite, and its rearing in the insectary has been difficult in almost every respect.

Mitchell and Mau (1971) presented data to support the theory that male N. N viridula produce a pheromone that is highly attractive to females of the same species and to the parasite N. N pennipes, and they believed that one explanation for the higher number of parasite eggs on the male stink bugs could be the response of the tachinid parasite to the male pheromone. Harris and Todd (1980a) also studied this chemical interaction between N, N viridula

and <u>T</u>, <u>pennipes</u>. They concluded that <u>N</u>. <u>viridula</u> males are the source of intraspecific attraction in this species, and observational data suggest the existence of a close-range, sexual communication mechanism, that may be pheromonal, but is more likely stridulatory. The second conclusion drawn from their experiments is that male and V instar <u>N</u>. <u>viridula</u> are attracted as strongly as female <u>N</u>. <u>viridula</u> to males of the species. These findings, they believe, strongly support the hypothesis that an olfactory substance is released by male <u>N</u>. <u>viridula</u> which, rather than being directly sexual (i.e., a sex pheromone) as reported by Mitchell and Mau (1971) acts as an aggregation pheromone for males, females and V instars of the species. The third conclusion is that female <u>T</u>. <u>pennipes</u> are much more strongly attracted to male than to female <u>N</u>. <u>viridula</u>.

Jones (1918) reported 25% of field collected N. viridula were parasitized (or with eggs on the body surface) by T. pennipes and Drake (1920) reported 10 to 80%, Bradley (1939) 40 to 45%; Todd and Lewis (1976) from 35 to 44%; Menezes et al. (1985) from 34 to 59%, Harris and Todd (1981b) and McPherson et al. (1982) from 14 to 40%.

Harris and Todd (1981b) concluded that the simple method of using the presence of parasite eggs on the host cuticle to estimate percentage of parasitization of field population of  $\underline{N}$ .  $\underline{\text{viridula}}$  by  $\underline{T}$ ,  $\underline{\text{pennipes}}$  is valid as a sufficiently good estimate of the true percentage of

parasitization. The percentages of wrong designations were 16.8% with parasite eggs and 16.6% without.

Harris and Todd (1982) developed a comparative study of field collected N. viridula and showed that parasitization by T. pennipes caused a 49% reduction in the longevity of male and female N. viridula and egg fertility and egg mass size were not reduced by parasitization. Fecundity of parasitized females was not reduced relative to that of unparasitized females during a time period equal to the lifetime of the parasitized female, but the lifetime fecundity of unparasitized females was 3.8 times the lifetime fecundity of parasitized females.

Harris and Todd (1982) concluded that since  $\underline{N}$ .  $\underline{viridula}$  mate and oviposit throughout their lives, parasitization by  $\underline{T}$ .  $\underline{pennipes}$  can cause significant reduction in population levels of this pest. This tachinid species has been recognized for many years as a natural control factor against populations of the southern green stink bug.

Capeluto (1949) considered  $\underline{T}$ . pennipes to be relatively ineffective as a biological control agent against  $\underline{N}$ .  $\underline{Viridula}$  populations for two reasons: they will deposit more than one egg per host individual, although only one larva can live to maturity, and  $\underline{N}$ .  $\underline{Viridula}$  continues to mate and oviposit despite being parasitized. Shahjan (1968) and Shahjahan and Beardsley (1975)

demonstrated that superparasitization is adaptative for  $\underline{T}$ .  $\underline{pennipes}$ , since first instar larva penetration of the host cuticle is only 50% successful.

Hokkanen (1985) discussed the 200 years of coevolution between N. viridula and T. pennipes and mentioned that N. viridula supposedly came to the Americas about 100-200 years ago, and then met the parasite, which does not occur outside the Americas, for the first time. The parasite quickly adapted itself to the new host; at least now it is considered to be the most important adult parasite of the southern green stink bug according to several authors (Drake 1920, Todd and Lewis 1976, Harris and Todd 1981b, McPherson et al. 1982).

Askew and Shaw (1986) discussed an alternative or a complementary classification of parasites, beyond the usual ecto and endoparasite. They called these idiobionts and koinobionts. In koinobionts they included most endoparasites of larvae and of adult insects, and the main characteristic is that the parasitized host continues to be mobile and able to defend itself; larval hosts are often not killed until they have prepared cryptic pupation retreats. The host may not live very long after parasitism, but the cardinal point is that koinobiont benefits from the continued life of its hosts. Idiobionts include the many ectoparasites which permanently paralyze or kill the host before the egg hatches. The host is

consumed in the same location and state in which it was attacked.  $\underline{T}$ ,  $\underline{pennipes}$  seems to be well categorized as a koinobiont species.

## Note

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# CHAPTER III TECHNIQUES FOR REARING <u>NEZARA VIRIDULA</u> AND <u>TRICHOPODA PENNIPES</u> AND CHARACTERIZING PARASITIZATION BY TRICHOPODA PENNIPES AND TRISSOLCUS BASALIS

#### Introduction

Progress and success in several areas of entomological research depend on the successful rearing of insects in the laboratory. The science (and art) of insect rearing has increased in complexity and sophistication during the last two decades. Early entomologists performed elegant studies using insects collected from the field or they reared insects on natural hosts. But new needs, such as mass production of parasites and predators, require many more insects. Artificial diets and controlled and manipulated environments have been developed, and these new techniques are currently very successful.

#### Rearing of Nezara viridula and Trichopoda pennipes

A considerable number of various life stages of Nezara viridula and Trichopoda pennipes were needed at various times to perform several experiments. Maintainance of strong laboratory colonies was necessary to provide parasitized and unparasitized bugs of the desired age. Field collected bugs could not be used because their ages and state of parasitism could not be determined without injury to the insects.

#### Parasitization by Trissolcus basalis

Nezara viridula egg masses turn gradually from darkyellow to gray and then to black when parasitized. These colors may suggest a true parasitization of the eggs.

Sometimes egg masses were already "hatched" when taken from the field and it was difficult to determine whether eggs were parasitized or unparasitized. The shape of the operculum opening can be used to determine parasitized eggs. Wilson (1961) stated that T. (Asolcus) basalis adult emerges through a round hole in the operculum. Hokyo and Kiritani (1966a) distinguished Asolcus mitsukurii Ashmead from Telenomus nakagawai Watanabe (Hym.:Scelionidae) according to shapes of the operculum opening (exit/emergence holes) of these two species. Each species, according to Hokyo and Kiritani (1966a), has only one characteristic emergence-hole shape, serving to distinguish between these two multiparasitic species.

I carefully tried to characterize  $\underline{T}$ .  $\underline{\text{basalis}}$  emergence hole shapes in the  $\underline{\text{N}}$ .  $\underline{\text{viridula}}$  eggs, during 1986 and 1987 egg parasitism observations, because shapes were not described in the literature.

#### Parasitization by Trichopoda pennipes

True parasitization of N. viridula adults by T.

pennipes has been confirmed when the host has a sclerotized tracheal funnel (Harris and Todd 1981b, 1982) or by the internal presence of a parasite larva (McPherson et al.

1982). Harris and Todd (1981b) stated that absence of a sclerotized tracheal funnel is usually sufficient evidence for declaring absence of parasitization. However, during preliminary observations to acquire experience with this parasite and host species, I observed that both mentioned evidences may be absent when a host was truly parasitized, producing a  $\underline{T}$ .  $\underline{pennipes}$  puparia. Due to this fact, I investigated other methods that might improve correct determination of  $\underline{N}$ .  $\underline{viridula}$  adult parasitization by  $\underline{T}$ .  $\underline{pennipes}$ .

To confirm parasitization of  $\underline{N}$ .  $\underline{viridula}$  by  $\underline{T}$ .  $\underline{pennipes}$  each host was divided into two halves along the pleural region from the posterior end of abdomen to anterior end of the thorax. Using dissecting forceps and a needle, internal tissues were separated permitting searching for parasite larva and/or its signal or evidence in the host body.

#### Materials and Methods

### Nezara viridula Rearing

The currently used laboratory food for the southern green stink bug is green beans, usually supplemented with raw shelled peanuts (Jones 1985, Harris and Todd 1981a). Although, Brewer and Jones (1985) tested and did not find a significant difference when rearing N. viridula on the above mentioned diet and on the meridic Debolt diet, no

mention was found of the latter artificial diet being utilized for rearing the southern green stink bug.

Sailer (1952) used Mason jars (home-canning glass jars) with gauze or paper toweling as lids, and green beans and shelled peanuts as food for rearing N. viridula for several years with apparent success, in small quantities. This method was utilized at first but air flow was too restricted and glass jars were replaced by screen (2 mm mesh) cylinders (90 x 150 mm), about the same volume as the Mason jars. Two plastic petri dishes (90 mm in diameter) were used as lids. Green beans and raw shelled peanuts were used as food.

Drastic changes in nymph rearing procedures were made compared to previously mentioned rearing methods. Fresh egg masses (2-4) were put in a small plastic petri dish (15 x 90 mm) with a water saturated dental cotton roll only. At 27° C, 70-80% RH and 14 h photophase, nymph emergence occurred in 4-5 days, and normally 2 days after emergence all nymphs moved from egg shells to the cotton roll, remaining aggregated. As nymphs molted to second instar, they were transferred to a larger plastic box (100 x 260 x 320 mm). The nymphs were not handled during transfer. The petri dish was inverted over the box and tapped vigorously knocking the nymphs into box. No mortality due to this procedure was ever observed. Pole bean pods and shelled peanuts were offered as food. The pods (2-3) were grouped

and held together by a rubber band, and peanuts placed inside a petri dish (90mm). This procedure saved a considerable amount of time and drastically reduced mortality compared to isolated pods and peanuts placed on the bottom of the boxes (as used by Harris and Todd 1980b). Every 3-4 days beans were changed for fresh ones and boxes cleaned. The various instars were maintained separately in individual boxes. For the V instar, large strips of paper were hung on the lid of the box, in order to provide a vertical surface for the final molt. This substrate was fundamental for successful molting; otherwise mortality due to malformed adults unable to shed (release) the exuvia was enormous.

Adults were transferred to the aforementioned cylindrical screen cages. A small tissue paper (Kimwipes<sup>TM</sup> 5 x 8.5 in) was hung inside each cage, held in place by the petri dish lid, providing the substrate for egg laying. These papers were checked and replaced daily. Egg masses were removed by cutting the paper around the mass, and stored or used to maintain the colony in progress. Adult food consisted of pole bean pods and shelled peanuts. Handling of insects was as minimal as possible during all phases.

To introduce native genes to laboratory colonies, fertilized females were frequently brought from the field and introduced into the oviposition cages. It was assumed

that this simple procedure would avoid or reduce inbreeding.

#### Trichopoda pennipes Rearing

Dietrick and van de Bosh (1957) described details of insectary propagation of <u>T. pennipes</u>, and their objective was to develop mass rearing techniques for this parasite using <u>Anasa tristis</u> (DeG.) as the host. My objective was to develop and maintain a small laboratory colony of parasites that could be used to parasitize <u>N. viridula</u> in the laboratory when needed for field experiments. The rearing of <u>T. pennipes</u> was simplified from the previously mentioned reference.

Two pairs of sexually mature T. pennipes, 1-2 days old, were placed in a large plastic petri dish (25 x 150 mm) containing about 15-25 N. viridula adults. Food for the flies consisted of a 20% solution of honey water, provided through a saturated dental cotton roll, and for the bugs one fresh pole bean pod. The petri dishes were held at 45° inclined position, under a 15 watt fluorescent light. The position of the petri dish prevented flies becoming stuck in large drops of bug secretions. The continuous light kept the flies active. Soon after being released in the cages flies started searching for the bugs and commenced oviposition. Trichopoda pennipes adults have an interesting behavior when confined. They lay motionless on their dorsum and only when disturbed, do they move, either

flying or walking. Flies were commonly found stuck on the cage bottom, destroying large portions of their wings. The inclined petri dish position resolved this most limiting problem.

Parasitized adults, with eggs on the body surface, were removed every other day, depending on their intended use. These insects were transferred to a "double cage." made with two similar plastic boxes (100 x 260 x 320 mm). One box was inserted in the other. The bottom of the top box was replaced with screen (5 mm mesh). The lower box was intact, and contained sand and vermiculite mixture. The screen bottom permitted only the parasite larva to fall through and pupate in a 2 cm layer of 1:1 mixture of washed sand and vermiculite which was maintained slightly wet. Nezara viridula parasitized adults were placed in the top cage and given pole or green bean pods for food. Every other day, dead bugs were removed, the top box was cleaned and puparia were removed from the sand and vermiculite substrate. The puparia were placed on a layer of tissue paper in a plastic petri dish (25 x 250 mm) until flies emerged. Every morning these petri dishes were atomized (sprayed) with tap water to maintain higher humidity, even though they were stored in a rearing chamber at 27°C, 70-80% RH and 14 h photophase. In the beginning a mixture of washed sand and vermiculite was used but adults emerged

malformed and at lower numbers compared to when paper was

Flies being used for parasitization of bugs were left in cages until their death and then removed. If necessary, flies were added without regards to number. Bugs were not replaced, but new cages were set up. Cleanliness of the petri dishes was found to be very important to maintaining flies in good condition and increasing longevity.

#### Results and Discussion

#### Rearing of Nezara viridula and Trichopoda pennipes

Changes undertaken on rearing methods previously mentioned permitted production of sufficient  $\underline{N}$ .  $\underline{viridula}$  life stages and adults parasitized by  $\underline{T}$ .  $\underline{pennipes}$  whenever necessary.

Egg masses were in the most demand and more than 500 were produced, and about 400 used to perform field experiments. An undetermined number of laboratory reared nymphs and adults were used, and a small proportion of the laboratory parasitized N. viridula adults were used. Procedures adopted in these rearing methods were successful in achieving the desired objectives.

# Trissolcus basalis Parasitization

During 1986 and 1987, about 150 parasitized egg masses (from laboratory and field) were observed, and after species confirmation, the shape of the operculum opening was examined and matched against 10-15 different shapes

previously noticed and drawn. It was possible from these to characterize the most common operculum opening shapes made by <u>T. basalis</u> and "emergence holes" made when eggs are located at the margin of the mass or when irregular rows and/or layers of eggs are deposited on the mass.

Occasionally eggs were found laid on top of other eggs and the parasite emerged through the top or by the side of the

The adult begins by gnawing at the center of the operculum, but the direction it takes, as well the size of the hole, does not seem to be uniform and symetric (as proposed by Wilson 1961), but irregular. If a part of the operculum remains, it is a parasite emergence hole.

It may be possible through a broad and careful study, to characterize, at least to family level,  $\underline{N}$ .  $\underline{viridula}$  egg parasite species using their pattern of emergence holes. This may be particularly important in field evaluation of egg parasitism when egg masses are usually taken with eggs already hatched and/or parasites emerged. This work has to be done for  $\underline{N}$ .  $\underline{viridula}$  egg parasites in the United States and elsewhere.

#### Trichopoda pennipes Parasitization

egg.

It is necessary to be able to separate signal from evidence of parasitization in <u>N. viridula</u> adults. Signals, are those that may suggest parasitization but not really prove such a state, for example, tachinid egg(s) on host

body surface, maggot penetration hole in the cuticle, flesh decomposed and rotted, red or purple coloration inside the abdomen, integument transparent on the abdomen. On the other hand, evidences are those that prove a true parasitization such as parasite larva inside the host body, sclerotized tracheal funnel produced by a tachinid larva, and abdominal cavity hollowed, associated with some signals mentioned, especially tachinid eggs and a penetration hole on cuticle. A bug was considered truly parasitized when any of the three categories were found. These criteria were adopted and used throughout my studies.

# CHAPTER IV PARASITISM OF NEZARA VIRIDULA IN SEVERAL HOST PLANT COMMUNITIES IN ALACHUA CO., FLORIDA

#### Introduction

As discussed by Barfield and Stimac (1981), two characteristics of many insect pest species that make them difficult to manage are mobility and polyphagy. They observed that we must understand the mechanisms (system processes) governing the ecology and dynamics of such species as a prerequisite for formulating strategies against them.

Stimac and Barfield (1979) developed conceptual models for a systems approach to pest management in soybeans. They stated that management of pests in the soybean system requires that pests be considered as part of the system or as inputs into the soybean system. This conceptual model hypothesized how pest problems in soybeans are influenced by other components in the agroecosystem, considering flows of pest inocula into a particular soybean field.

Migratory pests enter the target ecosystem from an unspecified source. This source is an input into a regional pool of pest inocula. The regional pool provides inputs of pest inocula into different areas, each of which can be viewed as an agroecosystem, containing various crop and non-crop habitats for the pest species. Pest inocula

in a given area are distributed into crop and non-crop habitats.

Stimac and Barfield (1979) stated that inputs of pest inocula can be from the area pool, local dispersal of pests between fields and resident pest populations; these inputs specify the potential for pest problems within any given soybean field site. These authors emphasized that the systems approach to pest management in soybeans implies that management strategies consist of tactics that are implemented in soybean fields as well as other fields and habitats providing inputs of pest inocula into soybean fields.

Nezara viridula is an extremely polyphagous species, particularly attracted to leguminous plants. In north-central Florida, either as a garden or field crop, several kinds of beans are grown throughout the spring, summer and fall, immediately before and after the soybean growing period. These sites are believed to be alternate hosts (pool of pest inocula) for N. viridula prior to entering soybean fields. Jones and Sullivan (1982) pointed out that even though many stink bug pests can feed and be maintained on a wide variety of plants, they may require a specific sequence of host species in certain areas in order to reproduce and knowledge of these key spring and summer hosts could enable alternate control strategies. Todd and Herzog (1980) presented a very interesting generalized

scheme for a typical seasonal host sequence of  $\underline{N}$ .  $\underline{viridula}$  in the southeastern coastal plain of the United States. When active during warm periods in winter they can be found feeding primarily on crucifers and small grains. As the weather begins to warm in the spring, the adults move into clover, early vegetables, corn, and tobacco where they feed and oviposit. The resultant nymphs and adults constitute the first spring generation. Tomatoes, leguminous and cruciferous vegetables, and okra become attractive in April, May and June, and these provide the major food source and oviposition sites during early and mid summer. By the time soybean becomes attractive in late July and August, third generation adults are present and invade soybean fields, which provide the only major source of food in late summer and early fall.

The questions relevant to answering what level of influence alternate host sites (host plant communities) might have for augumenting (enhancing) N. viridula populations in soybean fields are: which natural enemies are present, and at what level are they controlling the target pest in such alternate hosts, especially just before the soybean season? Are these alternate host sites favorable or unfavorable for potentially increasing N. viridula populations to further invade soybean fields? Are these sites desireable as a potential augumentative sites for N. viridula indigenous and exotic natural enemies?

To determine parasitic fauna of  $\underline{N}$ .  $\underline{viridula}$  on hosts that represent a real field situation in a given area, at least two major criteria should be met. First, the sampling period should be such to represent the majority of the entire period during which  $\underline{N}$ .  $\underline{viridula}$  is active, and secondly, that the samples taken represent the insect host population present during the period covered. Although it seems to be an easy task to meet these two criteria, it is not when faced with immeasurable problems associated with sampling commercial fields throughout a large geographical area.

The main objective of this work was to make a large number of observations on the most common spring and summer hosts, host plant communities including soybeans, to find out what parasites are present and the levels of parasitization on various  $\underline{N}$ .  $\underline{V}$  viridula life stages.

Todd and Herzog (1980) listed several factors that influence or dictate the number of sampling units that must be taken to answer questions about a population: 1) the reason for sampling, 2) the sampling distribution, 3) the true population mean, 4) the level of precision desired, and 5) the sampling method used. There is no doubt that when uncontrolled field situations are faced, the sampling method used must consider many pratical constraints. For example, when samples are to be taken in a private commercial field or from a crop of high value, the number

of samples taken may be fewer than ideal. Another important factor that directly influences the number of sampling units is the the reason for sampling. To document the occurrence of parasitism and to estimate levels of parasitization, samples may be taken for only the target species, for the life stage(s) which can be potentially parasitized.

#### Materials and Methods

To select sites for sampling, the following criteria were adopted: a) a planted area of at least .5 ha of the crop species to be sampled, b) permission for continuous sampling, c) ability to sample at least twice at 2 weeks intervals, d) sites located 5 to 7 km from the closest other field selected for the same crop species.

The 22 fields selected during 1986 and 1987 were located in Alachua Co., Florida., as seen in Figure 1. These fields were composed of 13 species of plants (Table 1). Specific locality, host plant composition in the agrosystem where the sampled crop was present, host sampled, approximate area occupied by the sampled crop, period of sampling, and the number of times the area was sampled are presented for each field site in Table 2.

Considering the previously mentioned potential problems for sampling under private field conditions, hand picking of insects for a fixed period was found to be the most viable method to standardize the sampling.



Figure 1. Location of host plant communities sampled in Alachua County, FL.

Table 1. Host plants sampled in Alachua Co., 1986 and 1987. N=number of fields.

Abbreviation	N	Common name	Scientific name		
CW	11	Cowpea	Vigna unguiculata (L.)		
C	3	Corn	Zea mays L.		
LB	6	Lima bean	Phaseolus lunatus L.		
T	1	Tomato	Lycopersicon esculetum Mill.		
G	3	Green beans	Phaseolus vulgaris L.		
S	4	Snap beans	Phaseolus vulgaris L		
E	1	Eggplant	Solanum melongena L.		
SF	1	Sunflower	Helianthus annus L.		
0	1	Okra	Hibiscus esculentus L.		
CA	2	Cabbage	Brassica o. var acephala		
W	1	Wheat	Triticum aestivum L.		
L	1	Lupine	Lupinus angustifolius L.		
SB	2	Soybean	Glycine max (L.) Merrill		

Table 2. Host plant fields sampled in Alachua Co., 1986 and 1987, N=number of samples.

Site	N	Locality	Crop composition	Approximate area (ha)	Dates sampled
Year:	1986				
1	4	Gainesville	CW/Cb	2	6/27-7/22
2	2	Melrose	CW/LB	0.5	7/9-19
3	4	Archer	CW/S/T	1	6/21-7/24
4	3	Windsor	CW	1.5	7/9-8/7
5	5	LaCrosse	CW/S/LB	8	7/14-8/20
6	2	LaCrosse	E	4	7/22-31
7	2	Newberry	SF	2	6/17-25
8	4	Gainesville	CW/L/G	2	6/21-9/6
9	5	Newberry	C	10	6/12-7/8
10	9	Newberry	CW/O/T	2	6/12-7/26
11	6	Archer	CA	1.5	5/7-6/16
Year	: 1987				
12	13	Newberry	CA	1.5	5/1-6/17
13	8	Newberry	W	12	4/12-5/11
14	7	LaCrosse	CW/LB/S	8	5/27-7/23
15	7	Newberry	CW/G	1.5	6/4-7/21
16	4	Newberry	C	2	5/20-6/6
17	8	Newberry	CW/G/LB	7	5/15-7/7
18	9	Newberry	CW/S	1	6/2-7/28

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Table 2 continued.

Site N		Locality	Crop composition	Approximate area (ha)	Dates sampled	
19	4	Newberry	С	1.5	5/18-6/10	
20	5	Gainesville	e L	7	3/9-4/1	
21	14	Newberry	SB	14	7/9-9/24	
22	21	Newberry	SB	4	7/27-10/22	

a) See Figure 1 for location on the Alachua Co. map. b) See Table 1 for name description.

Two additional factors were fixed: the same person sampled, and one hour of sampling time at each sampling site on each sample date was used throughout the period surveyed. This sampling program was designed to determine the presence of parasites and their relative levels of parasitization of  $\underline{N}$ .  $\underline{Viridula}$  rather than to estimate population densities of the parasites or bugs.

Nymphal instars IV and V and adults were collected from the plants throughout the area, without any special criteria. Whenever found, bugs were collected and stored together in a screen cage for transportation to the laboratory. Adults were randomly separated in groups of about 15 specimens and placed in large plastic petri dishes  $(25 \times 150 \text{ mm})$ , containing several tiny holes in the lids for ventilation. A large pole or green bean pod was supplied as food. This pod was replaced by a fresh one every 3-4 days. Nymphs were separated in the same manner, but sorted by instar. Petri dishes containing adults and nymphs were kept in a rearing chamber at 27°C, 70-80% RH. and 14 h photophase, and observed daily until their death. Dead insects were removed and under a microscope, each cadaver was examined. Tachinid eggs on body surface were counted and sex and parasitization were determined.

During the 1987, host plant community surveys, searching for N. viridula egg masses was included in order to determine egg parasitism. This searching was completely

casual, both within the field and within the plants. No special criteria, like searching time, area covered, number of samples, etc. was fixed, but whenever an egg mass was found, it was taken to the laboratory for evaluation. The main reason for such procedure was that the objective was not to estimate N. viridula egg density in these alternate hosts, but only to determine the presence of parasite species and its performance as a direct control agent. When searching for nymphs and adults, leaves were searched to locate egg masses, and those found were brought to the laboratory where their condition was noted. Egg masses were classified into one to five groups: 1. yellow-fresh, visibly very fresh, shining eggs; 2. yellow-old, not shining, but opaque and darker than yellow-fresh; 3. hatching, reddish eggs present, more than 50% of the eggs in the mass; 4. dark black or near black hatched eggs with opened operculum by nymphs or parasites.

The number of eggs per mass was counted, and then the mass was placed in a plastic petri dish (15 x 90 mm) containing a water saturated dental cotton roll to provide high humidity. Masses were stored in a rearing chamber at 27°C, 70-80% RH and 14 h photophase until nymphs or parasites emerged. When nymphs and/or parasites emerged, they were counted and parasites were stored in vials with 70% alcohol and saved for identification. Identification of the species was done by the author by comparing with

species identified by Dr. R. I. Sailer and confirmed by Dr. L. A. Stange.

#### Results and Discussion

Identification of parasites and estimation of the percentage of parasitization in the sampled host populations were the major objectives. It is necessary to recognize several potential problems and sources of bias in estimating percentage parasitism. These include 1) dead or injured hosts collected from the field dying soon after being taken to the laboratory; 2) interruption of exposure to parasites; 3) restriction of parasitism to certain stage, age, sex; 4) competition with other parasites or diseases; 5) bias due to changes in host longevity or behavior due to parasitism; 6) spatial variation of parasitism. To avoid such sources of error is difficult, because a detailed knowledge of the ecology, and behavior of parasite(s) and host is necessary. However, among these mentioned potential sources of bias, only three (1, 2 and 5 above) might have had some influence in these surveys, on the estimation of the parasitization levels. I assumed that bias 3 had no influence because hosts were collected completely at random, without any special criteria. Bias 4 was excluded because no other parasite or disease species were found (except in 1986, when Hexacladia hilaris occurred in two fields). Parasitism occurred throughout the area and period sampled whenever N. viridula was

present and for this reason bias 6 was not considered as a potential problem.

Thirteen different crop species were sampled as listed in Table 1, but in some areas cowpea was intercropped with other crops such as tomato, okra, lima beans, green beans, and snap beans. Samples were taken from these cowpea mixed crop areas such that the area was treated as a host plant community and insects were collected from the crop mixes. In the crop mixes, cowpea was always the largest crop in terms of area but occasionally N. viridula was collected in the other vegetables associated with cowpea in the mixed host plant community. No field with only tomato, okra, lima beans, green beans or snap beans was found of sufficient size by the criteria specified earlier.

Including the cowpea mix, a total of 9 types of host plant communities were sampled during 1986 and 1987.

Parasitism of Nezara viridula adults by Trichopoda pennipes was observed consistently in all host plant communities but parasitism by Hexacladia hilaris was rarely observed. H. hilaris was obtained only 6 times from N. viridula adults collected on host plants during the 1986 and 1987 surveys. H. hilaris was reared from 3 males and 3 females of N. viridula collected in cabbage, corn and cowpea mix in Alachua Co. (Table 3). However, in a total of 150 sample dates of N. viridula adults during 1986 and 1987, T. pennipes was

Table 3. Occurrence of  $\underline{\text{Hexacladia}}$   $\underline{\text{hilaris}}$  in  $\underline{\text{N}}.$   $\underline{\text{viridula}}$  adults in Alachua Co.

Date	Host sex	Host crop	Sample site		
5/26/86	Male	Cabbage	11		
6/23/86	Female	Corn	9		
5/30/87	Male	Corn	9		
5/30/87	Female	Cowpea mix	5		
5/20/87	Male	Cowpea mix	17		
5/20/87	Female	Cowpea mix	17		

a) see Table 2 and Figure 1 for site location.

present in every sample date (100%) and  $\underline{H}$ . <u>hilaris</u> was only observed in 4% of the samples.

During the two year period, 54.9% of all  $\underline{N}$ .  $\underline{viridula}$  adults collected were parasitized by  $\underline{T}$ .  $\underline{pennipes}$  and only 0.15% by  $\underline{H}$ .  $\underline{hilaris}$ . This indicates the extreme predominance of  $\underline{T}$ .  $\underline{pennipes}$  as the parasite of adult southern green stink bugs in the areas sampled.

Patterns of parasitization can be described in a variety of ways. They can be examined with respect to hosts insects, host plants of insect hosts or simply as temporal and spatial patterns. In the present study, the main interest was on factors influencing N. viridula parasitism in soybeans. Since patterns of parasitization in other N. viridula host plant communities may set the stage for parasitism in soybeans by determining the size of the parasite populations that can enter soybean fields, patterns of parasitization in the 9 different host plants were examined first. Then, spatial and temporal patterns of parasitization in the host plant communities were examined.

Parasitization is described by range, mean and standard deviation of the percent of  $\underline{N}$ .  $\underline{viridula}$  adults parasitized for females, males and total adults. Mean percent parasitization occurring during a time period can be calculated in different ways. The most common method of calculation is to divide the total number of individuals

parasitized by the total number collected times 100 for each site at which data are collected and then take the mean of the values for all sites. By this method each site represents one observation of percent parasitization. Another less common method of calculation is done when multiple samples are collected at each site during the season. With these data, a mean is calculated for each sample date at each site and the seasonal mean is calculated as the mean of all observation dates. The latter method of calculation produces estimates with larger standard errors because of the variability within season. In this study, percent parasitization was estimated by both methods and compared. The estimates of the means from the two calculations were not found to be statistically different (P>0.05). The results reported in tables are those from the latter method of calculation, using each sample date at each site as an observation. This is the more conservative way to examine differences between sites and crops because the standard errors of the means are larger than with other methods of calculation.

Five common host plant communities were sampled in both 1986 and 1987, but significant differences (P<0.05) in percent parasitization between years was found only for soybean (Table 4).

Table 5 summarizes mean percent parasitization for the 9 host plant communities for all samples taken in each

Table 4. Comparison of yearly mean percent parasitization of N.  $\underbrace{viridula}_{plant}$  adults by T.  $\underbrace{pennipe}_{plant}$  in four host plant communities, 1986 and 1987.

	Cowpea mix		Cabbage		Corn		Soybean	
Stage	1986	1987	1986	1987	1986	1987	1986	1987
Female	61.4ª	62.9a	33.4ª	46.7ª	59.8a	29.3ª	50.4ª	23.46
Male	73.9a	68.7ª	52.6ª	61.7ª	64.4ª	30.8ª	59.5ª	27.35
Adults	67.3ª	65.5ª	41.2ª	54.0ª	63.5ª	29.60	54.5ª	25.65

Means for each crop within a row followed by the same letter are not significantly different (P>0.05; Tukey's Studentized Range.

Table 5. Comparison of mean percent parasitization of  $\underline{N}$ .  $\underline{viridula}$  adults by  $\underline{T}$ .  $\underline{pennipes}$  in nine host plant communities, 1986 and 1987.

Female			Male			Total adults				
Hos	t na	range	mean	SD	range	mean	SD	range	mean	SD
CW	3	45.0	58.0ab	17.9	80.0	60.0ab	24.0	41.2	59.9ab	17.2
		78.6			80.0			75.0		
Cw	6	30.0	62.2ª	27.1	0.0	68.7ª	30.6	12.5	66.3ª	21.1
		100.0			100.0			100.0		
CA	20	12.5	42.00	22.2	25.9	58.5ªb	23.1	17.1	49.50	21.0
		88.9			94.7			89.9		
C	12	0.0	42.0ab	32.1	10.0	44.8ab	30.3	10.5	43.75	25.6
		100.0			97.1			90.3		
E	25	0.0	55.0ab	7.1	43.3	48.0ab	6.6	50.0	50.6ab	0.9
		60.0			52.6			51.2		
L	5	0.0	36.7ab	37.5	0.0	43.3ª b	25.8	20.0	43.8ab	26.2
		100.0			66.7			86.4		
SF	2	38.5	44.2ª b	8.2	64.7	82.3ab	24.9	53.3	62.4ª b	12.8
		50.0			100.0			71.4		
SB	26	0.0	33.85	28.4	0.0	34.1b	31.2	0.0	36.7b	27.6
		100.0			100.0			100.0		
W	8	18.2	44.0ab	20.5	50.0	78.9a	20.0	26.7	57.1ªb	20.4
		75.0			100.0			78.4		

a) number of sample dates

Means followed by the same letter in a column are not significantly different, (P>0.05; Tukey's Studentized Range.

plant community and compares levels of parasitization among

Cowpea mix showed the highest level of parasitization and soybean the lowest level for female, male and total adults. In all other host plant communities, levels of parasitization were generally intermediate between levels in cowpea mix and soybean. In sunflower, male mean percent parasitization was higher than in the cowpea mix but not statistically different (P>0.05) probably because the sunflower sample size was only two.

In cabbage, the percent parasitization of females and total adults was significantly (P<0.05) lower than the levels in the intermediate group. For females, the level was not statistically different from soybean.

In wheat, the percent parasitization of  $\underline{N}$ . viridula adult males was significantly higher than soybean and the intermediate host group and not statistically different (P>0.05) from the level of male parasitization in the cowpea mix.

In corn, the mean percent of adult parasitization was significantly lower (P<0.05) than cowpea mix and the intermediate host plant communities and not statistically (P>0.05) different than soybean.

The magnitude of the range and the standard deviation are values that may indicate the variability of a data set. For female, male and total adults collected from 9 host

plant communities during both years, the range of mean percent parasitization in each sample date had extreme values (0-100%), and females had more cases of extreme range than males and total adults. Maximum value of the range of parasitization (100%) occurred 4 times for each sex and twice for total adults. Cowpea mix and soybean were the two host plant communities in which 100% parasitization of total adults occurred during the sampling period. The narrowest range, which may suggest less variability in levels of parasitization, was found in eggplant.

Levels of parasitization at sites within an area can vary as a function of many abiotic and biotic factors other than the type of host plant community. Differences in percent parasitization between host plant communities were demonstrated but these differences could have been due to influences from site specific factors also. To examine site influences on levels of parasitization ANOVA and MRT were used to test differences between sites within each year. In 1986 and 1987, there were significant differences (P<0.05) between mean levels of parasitization at the different sites. These differences could have resulted from some influences of host plant community type. In order to partition the effects of host plant type from site, cowpea mix was examined because it was the only host plant community sampled at more than two sites in both

years. There were significant differences in levels of parasitization between sites in 1986 but not in 1987. In 1986, mean levels of parasitization for females and total adults were significantly different (P<0.05) between cowpea mix sites. However, there was no site difference in male parasitization (Table 6).

In 1986, site 2 had significantly lower parasitism of females than all sites, except site 8. Site 2 was located near Windsor, on the east side of Alachua Co., and fields with the host plant communities studied were extremely rare relative to other geographical areas covered in this survey. The lower percentage of parasitization may be due to a lower availability of hosts for <u>T. pennipes</u>. As a corollary, in areas where plant hosts are more abundant, more host insects develop and more parasites are present in the area.

Sites 1, 5 and 10 had significantly (P<0.05) higher levels of parasitization for females than for all sites except site 3, which was intermediate (Table 7). For total adults, site 1 is significantly (P<0.05) higher than all sites, except site 5. Sites 3, 5 and 10 are intermediate and site 2 is significantly lower than all other sites.

Table 7 summarizes  $\underline{T}$ . pennipes parasitization on  $\underline{N}$ .

Table 6. Comparison of mean percent parasitization of N. viridula adults by T. pennipes in cowpea mix communities, 1986.

Site	nª	Female	SD	Male	SD	Total Adults	SD
1	4	82.1ª	13.7	95.6ª	6.1	88.6ª	8.8
2	2	14.30	20.2	58.3ª	11.8	30.0d	14.1
3	4	57.2ªb	23.3	73.7a	37.7	61.3bc	25.9
5	4	84.9ª	12.7	75.0ª	21.5	83.3ab	13.7
8	4	35.9₺€	32.0	74.0ª	13.6	56.0cd	13.7
10	9	65.3ª	17.5	67.1ª	32.8	66.7bc	13.3

a) number of samples

Means in a column followed by the same letter are not significantly different (P>0.05; Tukey's Studentized Range.

Table 7. Mean percent parasitization of N. viridula adults by T. pennipes by site, 1986 and 1987.

			Female			Male		Tota	l adul	ts
Sit	e na	range	mean SD		range	mean	SD	range	mean	SD
1	4	62.7-	82.1	13.7	86.9-	95.6	6.1	77.7-	88.6	8.8
		94.7			100.0			96.5		
2	2	28.6-	14.3	20.0	50.0-	58.3	11.82	0.0-	30.0	14.1
		74.3			66.7			40.0		
3	4	27.3-	57.2	23.3	20.0-	73.7	37.7	25.0-	61.3	25.9
		84.0			100.0			86.2		
4	3	45.4-	58.0	17.9	33.3-	60.0	24.0	41.2-	59.9	17.2
		78.6			80.0			75.0		
5	4	72.2-	84.9	12.7	50.0-	75.0	21.5	70.0-	83.3	13.8
		100.0			100.0			100.0		
6	2	50.0-	55.0	7.1	43.3-	47.9	6.6	50.0-	50.6	0.9
		60.0			52.6			51.2		
7	2	38.5-	44.2	8.2	64.7-	82.3	24.9	53.3-	62.4	12.8
		50.0			100.0			71.4		
8	4	0.0-	35.9	32.0	7.1-	74.0	13.6	40.0-	56.0	13.7
		71.4			88.9			70.8		
9	5	31.6-	59.8	23.8	22.2-	64.4	30.9	40.9-	63.5	24.5
		88.9			97.1			90.3		
10	9	40.0-	65.3	17.5	0.0-	67.1	32.8	0.0-	66.7	13.3
		100.0			100.0			85.2		
11	7	19.7-	33.4	10.7	25.9-	52.6	20.4	6.3-	41.2	12.6
		50.0			80.0			57.9		

Table 7 continued.

		Female			Male			l adul	ts
Site na	range	mean	SD	range	mean	SD	range	mean	SD
12 13	12.5-	46.7	25.7	27.3-	61.7	24.6	17.1-	54.0	23.7
	88.9			94.7			89.9		
13 8	18.2-	44.0	20.4	50.0-	78.9	20.0	26.7-	57.1	20.4
	75.0			100.0			78.4		
14 9	36.4-	81.0	20.5	0.0-	63.7	39.3	43.7-	78.3	20.2
	100.0			100.0			100.0		
15 7	33.3-	51.6	11.1	0.0-	53.8	41.4	41.2-	54.2	10.1
	62.5			100.0			66.7		
16 4	14.3-	41.8	39.2	10.0-	20.2	10.9	20.0-	30.6	17.7
	100.0			33.3			57.1		
17 11	0.0-	54.6	36.1	18.2-	72.6	26.7	2.5-	62.2	27.4
	100.0			100.0			96.5		
18 9	28.6-	63.6	22.1	0.0-	65.2	30.9	41.7-	65.6	17.6
	100.0			100.0			100.0		
19 3	0.0-	12.8	12.5	20.0-	45.0	27.8	10.5-	28.2	15.7
	25.0			75.0			40.7		
20 5	0.0-	36.7	37.5	0.0-	43.3	25.8	20.0-	43.8	26.2
	100.0			66.7			86.4		
21 10	0.0-	50.4	29.1	0.0-	47.6	37.1	0.0-	54.5	28.7
	100.0			100.0			100.0		
22 16	0.0-	23.4	23.2	0.0-	25.6	24.6	0.0-	25.6	20.8
	62.5			66.7			61.1		
a) num	ber of	sample	s						

viridula adults for each of 22 sample sites during both years studied. Sites 1-11 and 21 were sampled in 1986 and sites 12-20 and 22 in 1987.

Details of  $\underline{N}$ .  $\underline{viridula}$  adult parasitization by  $\underline{T}$ .  $\underline{pennipes}$  and the incidence of tachinid eggs on the body surface are given in Appendix A.

 $\frac{Trichopoda}{Pennipes} \ was \ recovered, \ from \ all \ areas,$  where  $\underline{N}.\ \underline{viridula}$  was collected indicating that this indigenous parasite species is present throughout Alachua County.

Levels of parasitization were different for each sex. The mean percent parasitization for adult males was generally higher, regardless of host crop or site sampled (Table 7). In only 3 sites, mean percent parasitization for females was slightly higher than for males. The variability of parasitization was higher for females than males as indicated by larger standard deviation values.

The mean percent parasitization for total adults ranged from 25.6 to 86.6% during the period covered by this survey (March to October). At 16 of 22 sites (73%), mean percent parasitization for total N. viridula adult populations sampled were higher than 50%, indicating that T. pennipes is a parasite with "good" field activity. I use the term "good", because no other was found to better express parasite performance (or activity), when more than 50% of the host population is parasitized. I believe that

such a level, for a single parasite species, should be relevant, especially when the host is a mobile species present for only a short period in a given crop. Trichopoda pennipes was present and parasitized adult southern green stink bug at all sites when its populations reached levels where more than 2 individuals per sample were collected. Mean seasonal percent parasitization was used to examine spatial differences in parasitism of N. viridula with respect to host plant-types and site or geographical location. However, temporal patterns of parasitization within a season or year are also important in the evaluation of natural enemies. During the period, from March to October, host plant communities for N. viridula are always available in Alachua Co. and parasites can be collected or evaluated during this period. In March, only lupine was sampled and in April, only wheat. Levels of parasitization were generally lower than in the period May-July when cowpea and cowpea mix were sampled. All host plant communities sampled in August showed high levels parasitization. The level dropped in September and October when only soybean was sampled.

In 1986, samples were taken from May to September, and in 1987, from March to October. In 1986 and 1987, there were no significant differences (P>0.05) in mean percent parasitization among months. For 1986 and 1987, combining data, mean levels of parasitization were lower in May than

in June and July (Table 8). In 1986, percent parasitization of females and total adults reached a peak in August, but in 1987, no adult N. viridula were collected in August because soybean crop growth and pod formation were delayed due to a prolonged dry period. In September 1986, levels of parasitization remained high as in August, but in 1987, these levels were lowest in September and increased moderately in October.

Differences in levels of parasitization of  $\underline{N}$ .  $\underline{viridula}$  between years is influenced by which host plant communities are available and the relative abundance of each. For example, in August of 1987 and September and October of both years, the only abundant host plant community for  $\underline{N}$ .  $\underline{viridula}$  was soybean. Due to delay of pod set in the 1987 soybean crop, adults were scarce and consequently levels of parsitization were zero, and the lowest levels of parasitization of the entire period occurred in September and October (Figure 2).

Despite the fact that temporal patterns of parasitization between years are influenced by availability and condition of host plant communities, it is still useful to follow parasitization trends through the entire season. This, for example, may allow one to maximize collection of parasitized hosts in order to be used in other biological control activities such as shipment of natural enemies to other geographical areas or for inoculative of

Table 8. Monthly percent parasitization of N. viridula adults by T. pennipes in Alachua Co., 1986 and 1987.

			Female			Male		Total adults		
Mon	nª	range	e mean	SD	range	mean	SD	range	e mean	SD
Mar	4	0.0	39.6abcd	42.7	42.9	54.2ªb	10.2	25.0	49.8abcd	26.0
		100.0			66.7			86.4		
Apr	12	12.5	29.94	15.2	0.0	55.7ab	29.61	7.1	41.7acd	19.0
		52.9			100.0			75.0		
May	22	0.0	41.9abcd	25.6	0.0	54.9ab	31.1	10.5	47.6abcd	23.3
		88.9			94.7			89.9		
Jun	3	70.0	60.8ab	28.5	0.0	67.7a	30.4	26.3	65.2b	20.7
		100.0			100.0			100.0		
Jul	3	70.0	58.4ªb	27.4	0.0	64.1ªb	32.1	0.0	61.5bc	25.9
		100.0			100.0			100.0		
Aug	7	25.0	69.2ªc	24.9	0.0	48.8a b	34.6	20.0	69.0ab	24.7
		100.0			80.0			100.0		
Sep	15	0.0	33.3c d	22.9	0.0	45.5ab	34.9	0.0	31.8ªd	19.4
		63.2			45.5			61.1		
0ct	7	0.0	30.9abcd	23.6	0.0	30.96	18.1	0.0	39.4ª	23.8
		62.5			60.0			64.7		

a) number of samples. Means in a column followed by the same letter are not significantly different (P>0.05; Tukey's Studentized Range.

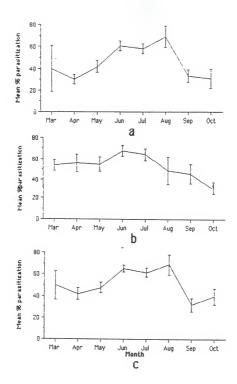


Figure 2. Mean percent parasitization of N. viridula a) females, b) males and c) total adults, for all samples, 1986 and 1987.

supplementary release in local areas where the parasite is not abundant.

Trichopoda pennipes is present and parasitizes  $\underline{N}$ .  $\underline{Viridula}$  adults when the host is present on early spring crops. The formulated hypothesis that parasitization levels increase during the spring and summer months and decrease late in the summer was confirmed. The same trend of parasitization levels was observed for females, males and total adults. However, females and total adults in March had higher levels than in April but the same finding did not occur for males.

As expressed in Figure 4, the standard error bars for female and total adult curves in March were the broadest, indicating a high variability of these levels. This trend of the parsitization levels, increasing during spring and early summer months, slightly decreasing during mid summer and drastically decreasing late in the summer was attributed also to changes in temperature. The increase of temperatures during the spring and early summer months, maintenance of high summer temperatures and much higher mid-late summer temperatures, respectively, could have dictated such parasitization trends.

Probably, time (temperature) and host plant availability and suitability are the factors that combine to give the best profitability for  $\underline{T}$ .  $\underline{pennipes}$  as a primary parasite for  $\underline{N}$ .  $\underline{viridula}$  adults in host plant communities

other than soybean. If this is true, when conditions are favorable to higher levels of parasitization of bugs in host plant communities prior to soybean pod set stage, fewer N. viridula adults (and probably nymphs) will enter in soybean fields during critical stages for damage by N. viridula. To test this hypothesis, experimentation over a wide geographical area would be necessary.

In biological control programs, we should not only be concerned with number of species present, and with better species to be used or introduced, but with how we can manage environmental factors (biotic/abiotic) to increase natural enemies profitability. During June, July and early August there is a significant parasite-induced mortality of N. viridula adults in cowpeas, consequently reducing the potential pest inocula for later planted soybeans. A possible tactic to reduce damage in soybean fields would be to augment T. pennipes parasitization by increasing the abundance of other N. viridula host plants near soybean fields. However, this hypothesis is still to be experimentally tested to determine if the benefits are greater than the negative effects.

The only species found parasitizing  $\underline{N}$ .  $\underline{viridula}$  nymphs was  $\underline{T}$ .  $\underline{pennipes}$ . Parasitization was extremely low (see Table 9). Of 130 nymphs (49 (IV) and 81 (V) instars) collected, only 4 (V instar) nymphs were parasitized by  $\underline{T}$ .  $\underline{pennipes}$ .

Table 9. Parasitization of N. viridula nymphs by T. pennipes, 1987.

	I	V instar	V instar			
Sex	Parasitized	Unparasitized	Parasitized	Unparasitized		
Female	0	23	1	49		
Male	0	26	3	28		

Four male (V instar) nymphs were collected and found to be parasitized; 3 T. pennipes puparia were recovered in the laboratory.

The egg parasite species which emerged from egg masses collected on host plant communities were <u>Trissolcus</u> basalis and <u>Ooencyrthus</u> submetallicus.

Fifty-three egg masses were collected on 4 host plants, from April 13 to July 9. These egg masses totaled 4416 eggs and 1008 eggs were parasitized (in 16 egg masses), giving parasitization of 22.8% for the period and crops sampled (see Table 10).

The highest level of parasitization was on corn (34.6%), followed by beans (25.2%). These two crops were sampled after May 18 and 30, respectively, and it may suggest that these parasite species become more active and/or abundant when weather warms. On May 1, one parasitized egg mass was collected for the first time on cabbage.

The egg mass itself may be of secondary importance considering that each egg in the mass is the unit that will generate new individuals. However, an egg mass has a reasonable proportion of its eggs parasitized, the remaining viable eggs which produce nymphs, should contribute little if any to a population increase. The reason for such speculation is apparently simple. At least in the laboratory, nymphs from a partially parasitized egg

Table 10.  $\frac{\text{Nezara viridula}}{\text{alternate host surveys, 1987. N-number, NP-number parsitized and %=percent parasitized.}}$ 

			Egg ma	sses			
Date	Crop	N	NP	%	N	NP	%
4/13	cabbage	1	0	0.0	69	0	0.0
20		4	0	0.0	292	0	0.0
27		2	0	0.0	151	0	0.0
5/01		6	2	33.3	380	89	23.4
6		1	0	0.0	77	0	0.0
11		1	0	0.0	95	0	0.0
Total		15	2	13.3	1064	89	8.4
4/13	wheat	1	0	0.0	79	0	0.0
5/6		1	1	100.0	82	14	17.1
Total		2	1	50.0	161	14	8.7
5/18	corn	1	1	100.0	78	67	85.9
30		2	0	0.0	188	0	0.0
6/02		6	62	33.3	501	155	30.9
7		2	2	100.0	158	152	96.2
10		2	0	0.0	157	0	0.0
Total		13	5	77.8	1082	374	71.0
5/30	beans	1	0	0.0	106	0	0.0
6/02		5	2	40.0	451	84	18.6
4		2	1	50.0	188	28	14.9
10		1	1	100.0	94	89	94.7

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Table 10 continued.

			Egg ma	sses	Eggs		
ate	Crop	N	NP	%	N	NP	%
11	beans	3	0	0.0	247	0	0.0
15		2	0	0.0	189	0	0.0
17		1	0	0.0	99	0	0.0
19		1	0	0.0	98	0	0.0
23		2	2	100.0	194	182	98.9
26		1	0	0.0	104	0	0.0
7/02		1	5	0.0	144	76	52.8
9		2	1	50.0	195	72	36.9
Total		23	8	65.0	2109	531	52.8

mass, did not aggregate in compact clusters on the egg shells as they do on "normal" hatched egg masses. If this also occurs in the field, we can assume that a much higher chance of mortality exists for fresh first instar nymphs, due to biotic (fire ants) and abiotic (rain) agents.

Compact aggregation in this instar was found to be adaptative and had an effect on the rate of development and mortality. In the laboratory, Kiritani et al. (1967) demonstrated that separation of individual nymphs from their egg mass siblings may provoke developmental differences, and Kiritani et al. (1966a) found that mortality decreased with an increase in the size of an aggregation.

CHAPTER V

POPULATION DEVELOPMENT OF <u>NEZARA VIRIDULA</u>, ITS PARASITISM AND EGG PREDATION IN SOYBEANS

## Introduction

In 1981, estimated losses plus cost of pest control in the southeast in soybeans totaled around 30 million dollars (Kogan and Turnipseed 1987). The southern green stink bug. Nezara viridula, is a major pest of soybeans in the southeastern United States (Kogan 1981). It is also a pest that may be disruptive to attempts to maximize the activity of indigenous natural enemies in soybean pest control as required in an integrated pest management program. Such disruptive effects follow when economically damaging populations of stink bugs are controlled primarily through the use of insecticides that have a broad spectrum of insecticidal activity. These insecticides reduce populations of beneficial insects and often lead to increased densities of several insect pest species. Use of these insecticides may have a tendency to become more intensive in soybean when the market value is low because they are cheaper. A means of controlling southern green stink bug that does not severely reduce populations of natural enemies would be helpful because the benefits from natural enemies for control of other insect pests such as the velvetbean caterpillar and soybean looper can reduce production costs.

In order to evaluate alternative pest management strategies for  $\underline{N}$ .  $\underline{viridula}$  an assessement of indigenous natural enemies is a prerequisite. The objectives of this research were to investigate  $\underline{N}$ .  $\underline{viridula}$  population dynamics and to describe parasitism and egg predation by fire ants in soybean in an Alachua Co. agroecosystem.

To investigate population dynamics and natural enemy effects, sampling strategies of different life stages must be formulated. Kogan and Herzog (1980), Todd and Herzog (1980) and Herzog (1984) discussed several aspects of sampling soybean insects and Herzog (1984) concluded that sampling has always been a controversial issue. He mentioned that probably the most provocative has been the choice of a sampling method for mobile life stages: the shake/beat cloth or the sweep net. A second controversial subject has been the need for quantification of absolute population densities because there are those who maintain that relative sampling methods provide all the information necessary. Herzog (1984) believes that the truth lies somewhere between the two extremes.

Schuman and Todd (1982) studying population dynamics of the southern green stink bug in relation to soybean phenology, found that direct measurements of oviposition in the field are very difficult to make, due to the large plant surface area which must be searched for egg masses, and in previous attempts to directly measure field

oviposition by N. viridula in soybean at Tifton, Georgia they failed. The dispersion of N. viridula egg masses in a field is primarily determined by the dispersion of the ovipositing females and by their degree of activity and pattern of movement (Todd and Herzog 1980). The only research concerning spatial patterns of stink bug species was conducted on rice in Japan by Nakasuji et al. (1965) who reported that under most conditions invading females of N. viridula disperse at random into rice fields. Todd and Herzog (1980) mentioned that a similar situation occurs in soybeans, because the dispersion of females and their redistribution is random, and egg masses are deposited at random, fitting the Poisson distribution (Hokvo and Kiritani 1963). Panizzi et al. (1980) studied the internal dispersion of N. viridula in a soybean field in Brazil, and concluded that the southern green stink bug moved more along than across rows, and the maximum distance recorded along the rows was 12 m, while across rows was 7.2 m from the release point.

Nezara viridula is an insect pest that has only two common parasite species occurring in soybean fields in the United States. In Florida, South Carolina, Georgia and Louisiana field studies have been conducted to evaluate parasitism and all reported results to date confirm only two parasites of N. viridula. Differences in natural enemy composition and density are often a dynamic temporal and

spatial situation. The meaning and significance of these differences may impact strongly on control options and upon the overall design of pest management strategies for soybean insects.

The impact of the red imported fire ant, <u>Solenopsis</u> <u>invicta</u> Buren on soybean insects has received significant attention, especially its predation on velvetbean caterpillar, <u>Anticarsia gemmatalis</u> Hubner (Elvin et al. 1983), and the southern green stink bug, <u>N. viridula</u>, (Ragsdale et al. 1981, Krispyn and Todd 1982, Stam et al. 1987).

Ragsdale et al. (1981) determined that southern green stink bug egg predation by S. invicts occurred during the entire soybean season, from plant developmental stages V-6 through R8 and it was an efficient predator. Stam et al. (1987) indicated that S. invicts was the major species preying on N. viridula egg masses in the soybean ecosystem in Louisians. Krispyn and Todd (1982) in caged experiments demonstrated the severe impact that S. invicts may cause on N. viridula population growth. In cages where red imported fire ants were excluded, the N. viridula population reached 7279 bugs and in those where the fire ants were present only 455 bugs developed during the same time period and conditions. They also stated that preferential predation was on nymphs and this fact may drastically enhance the impact of fire ants on stink bug population reduction,

because egg parasitism by scleniod and encyrtid species should be an important additive mortality factor normally found in a soybean field, at least in the southeastern United States (Orr et al. 1985, 1986).

## Materials and Methods

An experimental soybean field of 14.5 ha with variety Coker 237, maturity group VII, was planted on June 6, 1986 at David Hodge's farm, located in Newberry, Alachua Co. Florida. This field was planted and agronomically managed according to the usual farming procedures for soybeans in this region and no effort was made to modify any usual agronomic activities including pest control. The entire area was divided into 14 "plots" of one ha each. In each "plot," 3 random samples were taken using systematic sampling with a random start. A total of 42 samples were taken for each sampling date. Sampling began at growth stage V5 on July 9 (soybean growth stages were determined according to Fehr and Caviness 1980) and continued up to R7 on September 24. From V5 to R6, samples were taken once a week, and after September 4, twice a week (Monday and Thursday) until harvest.

The beat cloth method described in Kogan and Herzog (1980) was used for sampling nymphs and adults. Fourth and V instars and adults of N. viridula were collected and taken to the laboratory. First, II and III instars were counted, recorded and left in the same area.

The 1987 soybean field consisted of an uniform area of 20 ha, variety Bradford, maturity group VII, planted on June 14 and located in another area at David Hodge's farm in Newberry.

The field was planted and initially managed according to usual procedures of the farmer and those for soybeans in this region. Due to a prolonged dry period, the farmer almost abandoned the field. He did not use herbicide nor cultivate the area more than once for weed control. A large amount of weeds developed over most of the area. It was necessary to select an area of 4 ha (140 x 290m), located centrally in the field with very uniform plants for sampling. This experimental area was sampled using systematic sampling with a random start and 20 samples were taken for each sampling date. The beat cloth method for sampling was used as in 1986. Fourth and V instar nymphs and adults were collected but I, II and III instars were counted, recorded and left in the same area.

An egg density experiment was conducted during the 1987 soybean season in the experimental soybean field at the Hodge's farm. Observations started on July 27 (V-9) and continued until October 8 (R-7). Each observation consisted of carefully searching for N. viridula egg masses along one row meter of plants randomly located in the first 5 steps shead of the location where the beat cloth samples

were taken. The beat cloth samples were taken systematically as explained earlier.

Egg mass samples were taken by two persons, one on each side of the row, searching on and inside the plant canopy, searching all leaves and other plant parts, as well as the weeds present in the particular area. The beat cloth handle was used to determine the one meter area of plants. Whenever an egg mass was found, it was removed from the plant, put in a paper cup, numbered according to the sampling station number and was taken to the laboratory. In the laboratory, total number of eggs, number of normal hatched eggs, number of parasitized eggs were counted and recorded, and the condition of the egg mass was noted (yellow-fresh, yellow-old, dark, hatching, hatched). Each egg mass was put in a small plastic petri dish (15 x 90 mm) with a water saturated dental cotton roll to maintain high humidity and stored in a rearing chamber at 27°C, 70-80% and 14 h photophase, until the emergence of all nymphs and/or parasites.

Sampling for nymph and adults in 1987 began on July 21 at growth stage V-9 and continued to R-8 on October 22. From V-9 to R-4 on August 3, sampling was conducted once a week and from R-5 (Aug 7) to R-8 (Oct 22) twice a week (Monday and Thursday).

In 1986 and 1987,  $\underline{\text{N}}$ .  $\underline{\text{viridula}}$  IV and V instar nymphs and adults collected in the beat cloth samples were taken

to the laboratory and individualized in a small plastic petri dish (15 x 90 mm), and 1/3 of a pole or green bean pod was provided as food (replaced every 2-3 days) and held in a rearing chamber at 27°C, 70-80% RH, and 14 h photophase. Petri dishes were checked daily and the dead insects were removed. The dead insects were examined and sexed; parasitism, and number of tachinid eggs were determined. An insect was considered truly parasitized when any of three evidences were present, as explained before in Chapter III. When a tachinid puparium was present, it was maintained in the same petri dish, a water saturated dental cotton roll was added and maintained wet to increase humidity until the fly emerged.

When searching for N. viridula egg masses to estimate parasitism, plants were searched along rows and a thorough visual examination of leaf surfaces was conducted by bending the upper 1/3 - 2/3 of plants using a sweep net handle to bend back foliage allowing examination of leaf surfaces. Plants were bent to the left and right by the sampler to ensure that most leaves were examined. In both years, sampling was conducted twice weekly, and each sample date consisted of one hour of searching the plants. The sampling period was from July 25 (V-9) to August 22 (R-5), and July 28 (V-9) to October 13 (R-7), respectively, in 1986 and 1987. Sampling was conducted in several areas over the fields, but in 1986, it was concentrated in about

1/3 of the entire experimental area covering approximately 4 ha of the field. Collected egg masses were brought to the laboratory where their condition was noted using the same criteria adopted for egg masses collected in hosts other than soybean. Numbers of eggs were counted and then each egg mass was isolated in a small plastic petri dish (15 x 90 mm) containing a water saturated dental cotton roll and held in a rearing chamber at 27°C, 70-80% RH, and 14 h photophase until all parasites or nymphs emerged. Host eggs that remained intact for 3 weeks after collection were dissected to determine whether or not they were parasitized.

Parasite adults were counted and stored in vials with 70% alcohol for future identification. Each egg mass was evaluated for number of naturally hatched and unhatched eggs and emerged parasites.

An egg predation experiment was conducted in the same area under soybean field conditions similar to those described earlier. Laboratory laid egg masses were used to insure that egg masses would be available during the entire soybean growing season. In 1986, fewer egg masses were required, consequently fresh egg masses (4-7 days stored in a refrigerator at 8°C) were used, but in 1987 it was necessary to use egg masses stored for 1 to 2 months. Egg masses exposed in both years were yellow in color, with turgid eggs, although eggs in some masses may have been

preyed upon by adults while in the rearing/ovipositing cage. Only egg masses with a normal appearance (resembling those collected from the field) were exposed in predation experiments.

In 1986, the experimental area was 14 ha and there were 5 stations, one at each corner and 1 at the center of the field. Weekly two egg masses were exposed (10 egg masses/week/whole area) at each station from July 7 (V9) to September 1 (R6). The egg masses were glued to the underside of a soybean leaf located at about the center of the plant and outer edge of the canopy. The leaf containing the egg mass was marked using 3 to 5 round holes made with a paper punch. The location of the leaf was marked by placing a small flag in the ground near the leaf. Every 24 hours these leaves were observed to verify the condition of egg masses. Whenever an egg mass was found occupied by fire ants, the ants were collected and the egg mass was considered consumed. Otherwise, when at least 70-80% of the eggs in the mass were removed an egg mass was considered taken.

Weekly in 1987 at each of the 20 systematically selected sampling stations one egg mass was exposed (20 egg masses/week/area) and glued to the underside of a soybean leaf as was done in 1986. The leaf marking procedures were the same as those used in 1986. A small drop of Elmers Glue-All TM, was used on each leaf. A simple test was

conducted to determine if this glue might attract fire ants. A large drop was placed and periodically observed on leaves and on no occasion were ants or evidence of their presence on the drops found, and for this reason it was assumed that the glue alone was not attractive to fire ants.

All egg masses exposed were laid in laboratory on Kimwipes TM tissue paper. Paper was trimmed close to egg masses allowing some surface for gluing egg masses to soybean leaves. It was assumed that this minute piece of paper had no influence on fire ant attraction.

## Results and Discussion

In 1986, the seasonal mean numbers of N. viridula nymphal and adult specimens collected from beat cloth samples were distributed as follows: 0.42 adult (0.20 female and 0.22 male), 0.71 II instar, 0.35 III instar, 0.16 IV instar and 0.10 V instar nymphs (Table 11). The last sampling date was on September 24 (R7) because the field had to be sprayed to control an outbreak of lepidopterous defoliators. The farmer for economic reasons sprayed methyl-parathion which virtually killed all insects in the soybean field. At the time of the application plants were almost mature (senescent).

Table 11. Mean number of  $\underline{N}$ .  $\underline{viridula}$  nymphs and adults collected from 42 beat cloth (3 row ft each) samples for each date in soybean, 1986.

			Nyn	nphs			Adults	
Date	Growth stage	II	III	IV	V	Female	Male	Total
7/9	V5	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	V8	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	V1	0.00	0.00	0.00	0.00	0.05	0.00	0.05
30	R2	0.09	0.00	0.00	0.00	0.00	0.00	0.00
8/6	R2	1.09	0.00	0.00	0.00	1.09	0.02	0.12
15	R3	0.24	0.02	0.00	0.00	0.02	0.00	0.02
21	R4	0.83	0.12	0.00	0.00	0.00	0.00	0.00
29	R5	0.83	0.26	0.02	0.00	0.21	0.09	0.31
9/4	R6	0.50	0.36	0.12	0.00	0.14	0.02	0.17
10	R6	1.14	0.48	0.28	0.05	0.14	0.26	0.40
13	R6	1.17	0.86	0.28	0.26	0.45	0.31	0.76
17	R6	0.67	1.17	0.47	0.21	0.33	0.48	0.81
20	R6	0.48	0.52	0.28	0.50	0.64	0.64	1.28
24	R7	2.89	1.07	0.78	0.38	0.78	1.12	1.92
Season	nal means	0.71	0.35	0.16	0.10	0.20	0.22	0.42

In 1987, the seasonal mean numbers of N. viridula nymphs and adults collected were: 0.46 adult (0.25 female and 0.21 male), 0.29 II instar, 0.28 III instar, 0.10 IV instar and 0.24 V instar nymphs (Table 12). The field was sprayed once (August 27, R4) and the insecticide Dimilin TM was used. Since this product does not effect N. viridula life stages and natural enemies, it was assumed that in 1987, despite lower incidence of this pest, there was a natural occurrence of the host and natural enemies throughout the season.

Nymphs were first collected during soybean reproductive growth stages. In neither year was a first instar nymph collected in the beat cloth samples. In 1986, II instar nymphs were collected first on July 30 (R2), III instar on August 15 (R3), IV instar on August 29 (R5) and V instar on September 10 (R6) as seen in Figure 3a. This sequential occurrence of numphal instars may suggest that the first generation of N. viridula in soybean started in the R2 growth stage. In 1987, such sequential appearence of instars in a temporal distribution was not observed, probably indicating an early overlap of generations (Figure 3b). In both years, nymphal populations grew until mid-September to early October (R6 and R7 growth stage). In 1987, samples taken during mid-October (R8 growth stage) showed a severe decline in number of nymphs.

Table 12. Mean number of  $\underline{N}$ .  $\underline{viridula}$  nymphs and adults captured from 20 beat cloth (3 row ft each) samples for each date in soybean, 1987.

			Nyn	nphs	Adult			
Date	Growth stage	II	III	IV	V	Female	Male	Total
7/27	V9	0.00	0.00	0.00	0.00	0.05	0.00	0.05
8/03	R1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	R2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	R2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
24	R3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31	R4	0.00	0.10	0.00	0.00	0.00	0.00	0.00
9/03	R4	0.00	0.10	0.00	0.00	0.10	0.05	0.15
7	R5	0.00	0.00	0.00	0.05	0.10	0.05	0.15
10	R5	0.60	0.20	0.00	0.00	0.30	0.05	0.35
14	R6	0.20	0.05	0.05	0.00	0.40	0.25	0.65
17	R6	0.60	0.05	0.10	0.05	0.10	0.05	0.15
21	R6	0.55	0.00	0.30	0.10	0.10	0.15	0.25
24	R6	0.30	0.50	0.30	0.30	0.30	0.15	0.45
28	R6	0.30	0.35	0.25	0.45	0.25	0.25	0.50
10/1	R6	1.00	1.00	0.20	0.80	0.40	0.50	0.90
5	R7	1.05	0.85	0.00	0.30	0.65	0.50	1.15
8	R7	0.00	0.00	0.35	0.75	0.50	0.60	1.10
12	R7	1.25	0.40	0.40	0.35	0.85	0.60	1.45
15	R8	0.05	0.55	0.20	0.65	0.90	0.60	1.50
19	R8	0.15	1.10	0.10	0.85	0.35	0.60	0.95
22	R8	0.00	0.15	0.05	0.35	0.40	0.05	0.45
Season	al means	0.29	0.28	0.10	0.24	0.25	0.21	0.46

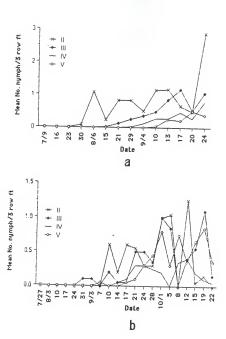


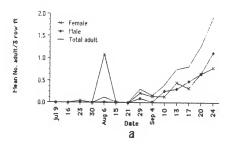
Figure 3. Mean number of N. viridula nymphs collected in soybean, a) 1986, b) 1987.

Perhaps this soybean stage is not suitable for nymphal feeding.

Adult females were the first N. viridula specimens collected in soybean during both years. In 1986, it was on July 23 (V10) and in 1987 on July 27 (V9). In 1986, the adult population peak occurred late in the season between September 4 (R6) and September 24 (R7), but in 1987 there was a small peak between September 10 (R5) and September 14 (R6) and the acme of the adult population occurred during September 28 (mid-R6) and October 19 (early R8) (Figure 4).

In 1986, 42 beat cloth samples were taken (1 m of row) on each of 14 sample dates, giving 588 row meters of plants sampled throughout the sampling period. In 1987, 20 beat cloth samples were taken on each of 21 occasions, giving 420 row meters of plants sampled. Average population (nymphs II - V and adults) per row meter of plants in 1986 was 1.73 individuals and in 1987 was 1.37. These figures indicated that relative population densities were almost equal in both years. Adult female and male numbers were practically equal in both years as indicated by the seasonal average sex ratios which were 1.02:1 and 1:0.84 female to male in 1986 and 1987, respectively.

Soybean agronomic management decisions are usually made on the basis of crop growth stage rather than on a calendar basis. For this reason, mean number of  $\underline{N}$ .  $\underline{viridula}$  IV and  $\underline{V}$  instar nymphs and adults were calculated for each soybean



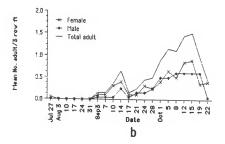


Figure 4. Mean number of N. viridula adults collected in soybean, a) 1986, b) 1987.

growth stage (Table 13). These means were calculated from the numbers of individuals captured per beat cloth sample for all dates within a growth stage. Mean number of IV or V instar nymphs was never higher than 0.76 ( $\pm$  0.19) nymphs per beat cloth for any growth stage during the 1986 and 1987 soybean seasons.

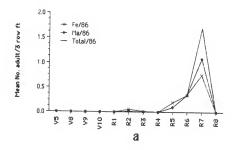
In 1986, IV instar nymphs were captured only during R5, R6 and R7 and V instars during R6 and R7 (last sample was late R7). The seasonal mean numbers of nymphal instars were practically equal. In 1987, the IV instar nymphal population was lower than V instar and both instars occurred from R5 to R8 soybean growth stages (Table 13).

The treatment threshold level for stink bugs, including the southern green stink bug, in commercial soybean fields in Florida, is an average of one per beat cloth (IV plus V instar nymph plus adult) prior to seed formation and 3 per beat cloth after this stage. By these criteria the threshold level was not exceeded during 1986 or 1987, although in 1986 bug density reached a value of 2.95.

In the 1986 and 1987 seasons, total mean numbers of adults (mean number of females and males) were nearly equal, 0.57 adults in 1986 and 0.58 adults in 1987 (Figure 5). As mentioned earlier in Materials and Methods, in 1986 insecticides were used twice but only the first application should have influenced the N. viridula population trend

Table 13. Mean number of  $\underline{\text{N. viridula}}$  IV, V instar nymphs and adults sampled in each soybean growth stage, 1986 and 1987.

	Ny	mphs		Adults	
Growth stage	IV	V	Female	Male	Total
Year: 1	986				
V5	0.00	0.00	0.00	0.00	0.00
V8	0.00	0.00	0.00	0.00	0.00
V10	0.00	0.00	0.00	0.00	0.00
R2	0.00	0.00	0.05 <u>+</u> .02	0.01 <u>+</u> .01	0.06 <u>+</u> .03
R3	0.00	0.00	0.02 <u>+</u> .02	0.0	0.02 <u>+</u> .02
R4	0.00	0.00	0.00	0.00	0.00
R5	0.02 <u>+</u> .02	0.00	0.19 <u>+</u> .07	0.09 <u>+</u> .06	0.28 <u>+</u> .10
R6	0.26 <u>+</u> .04	0.25 <u>+</u> .04	0.34 <u>+</u> .04	0.35 <u>+</u> .05	0.69 <u>+</u> .07
R7	0.76 <u>+</u> .19	0.38 <u>+</u> .09	0.74 <u>+</u> .13	1.07 <u>+</u> .16	1.81 <u>+</u> .24
Year: 19	987				
V9	0.00	0.00	0.00	0.00	0.00
R1	0.00	0.00	0.00	0.00	0.00
R2	0.00	0.00	0.00	0.00	0.00
R3	0.00	0.00	0.00	0.00	0.00
R4	0.00	0.00	0.02 <u>+</u> .02	0.07 <u>+</u> .05	0.10 <u>+</u> .07
R5	0.10 <u>+</u> .07	0.02 <u>+</u> .02	0.07 <u>+</u> .04	0.17 <u>+</u> .07	0.25 <u>+</u> .09
R6	0.22 <u>+</u> .06	0.28 <u>+</u> .08	0.22 <u>+</u> .04	0.24 <u>+</u> .04	0.46 <u>+</u> .07
R7	0.12 <u>+</u> .05	0.45 <u>+</u> .10	0.47 <u>+</u> .09	0.65 <u>+</u> .15	1.12 <u>+</u> .19
R8	0.12 <u>+</u> .05	0.62 <u>+</u> .11	0.42 <u>+</u> .10	0.55 <u>+</u> .11	0.97 <u>+</u> .17



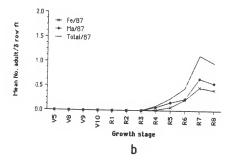


Figure 5. Mean number of <u>N. viridula</u> adults collected in soybean during each growth stage. a) 1986, b) 1987.

because the second application was after the last sample date. In 1987, it was assumed that N. viridula population growth was natural throughout the entire soybean season, for reasons explained earlier.

Seven egg masses were found during the 17 sample dates in 1987 and all were found after soybeans reached reproductive stage. Sample dates included all soybean reproductive stages and 340 row meters of plants (both sides) were examined. The first egg mass was found at R-5 (beginning seed) and thereafter almost consistently on every sample date one egg mass per sample was found (Table 14). No peak was observed because only once, on September 17, was more than one egg mass found.

The 7 egg masses had a total of 525 eggs with a mean of 75 eggs per mass. These low values may help to explain total absence of first instar nymphs in beat cloth samples since this instar remains aggregated on egg shells.

A much larger area in the soybean field was covered when egg masses were collected during one hour of visual sampling for egg parasitism determination. During the same period, 99 egg masses were found through the "walk-open-leaves" method. Total area of plants covered by these observations were several hundreds of times larger than those covered by one meter row. In the "walk-open-leaves" method, 32 man-hours were spent looking for egg masses. Ninety-nine egg masses were found giving an average of

Table 14. Egg masses collected to estimate  $\underline{\text{N}}.$   $\underline{\text{viridula}}$  egg density in soybean, Alachua Co.,  $\overline{1987}.$ 

Date	Growth stage	No. of samples	Egg masses	Total eggs	Egg mass condition
7/27	V9	20	0	0	-
8/3	R1	20	0	0	-
8/10	R2	20	0	0	-
8/17	R2	20	0	0	-
8/24	R3	20	0	0	-
8/31	R4	20	0	0	_
9/3	R4	20	0	0	-
9/7	R5	20	1	77	hatched
9/10	R5	20	0	0	-
9/14	R6	20	0	0	-
9/17	R6	20	1	81	hatched
9/17	R6	20	1	92	yellow-fresha
9/21	R6	20	1	96	hatched
9/24	R6	20	1	16	yellow-old
9/28	R6	20	1	108	hatched
10/1	R6	20	0	0	_
10/5	R7	20	1	55	hatched
10/8	R7	20	0	0	-

a) found on a weed leaf, Panicum sp.

3.09 egg masses (or 267.6 eggs) per hour of searching, during the entire sampling period.

The only N. viridula nymphal and adult parasite species found during 1986 and 1987 soybean surveys was Trichopoda pennipes. This species was practically always present in soybean fields during the two seasons when N. viridula was present.

In 1986, the seasonal average percentages of parasitization for adult females and males were 50.4% and 59.5%, respectively. In 1987, these values were 23.4% and 27.3%. The adult population was parasitized at an average level of 54.5% in 1986 and 25.6% in 1987 (Table 15 and 16).

Nymphs had a lower level of parasitization by T.

pennipes. Fourth instar nymphs were parasitized at mean levels of 4.2% and 2.4% in 1986 and 1987, respectively, and V instar parasitization was higher, 18.4% in 1986 and 10.0% in 1987. Figure 6 represents the mean percent parasitization for N. viridula adults on each sample date in both years. In 1986, a peak of parasitization occurred on August 15 (R3) but the estimated level of parasitization resulted from only one parasitized female. Obviously, this small sample size may not be representative of a parasitism trend. Parasitization of adults occurred consistently at levels of 48.1% to 84.6% after the R4 growth stage in 1986. In 1987, the range of parasitization was 15.8% to 61.1% after mid-R6 stage.

Table 15. Mean percent parasitization of  $\underline{N}$ ,  $\underline{viridula}$  nymphs (IV and V instars) and adults collected on each sample date in soybean, 1986.

			Nymphs		Adults			
Date	Growth stage	IV	v	Total	Female	Male	Total	
7/9	V5	0.0	0.0	0.0	0.0	0.0	0.0	
16	V8	0.0	0.0	0.0	0.0	0.0	0.0	
23	V1	0.0	0.0	0.0	0.0	0.0	0.0	
30	R2	0.0	0.0	0.0	0.0	0.0	0.0	
8/6	R2	0.0	0.0	0.0	25.0	0.0	20.0	
15	R3	0.0	0.0	0.0	100.0	0.0	100.0*	
21	R4	0.0	0.0	0.0	0.0	0.0	0.0	
29	R5	0.0	0.0	0.0	88.8	75.0	84.6	
9/4	R6	0.0	0.0	0.0	50.0	100.0	57.1	
10	R6	8.3	0.0	5.5	33.3	81.8	64.7	
13	R6	0.0	9.0	4.3	63.1	38.4	53.1	
17	R6	15.0	22.2	17.2	50.0	75.0	64.7	
20	R6	0.0	33.3	21.2	48.1	48.1	48.1	
24	R7	0.0	12.5	4.1	5.4	57.4	52.5	

<sup>\*</sup> based on one parasitized female

Table 16. Mean percent parasitization of N. viridula nymphs (IV and V instars) and adults collected on each sample date in soybean, 1987.

			Nymph	3		Adults	
Date	Growth	īv	V	Total	Female	Male	Total
7/27	V9	0.0	0.0	0.0	0.0	0.0	0.0
8/03	R1	0.0	0.0	0.0	0.0	0.0	0.0
10	R2	0.0	0.0	0.0	0.0	0.0	0.0
17	R2	0.0	0.0	0.0	0.0	0.0	0.0
24	R3	0.0	0.0	0.0	0.0	0.0	0.0
31	R4	0.0	0.0	0.0	0.0	0.0	0.0
9/03	R4	0.0	0.0	0.0	0.0	0.0	0.0
7	R5	0.0	0.0	0.0	50.0	0.0	33.3
10	R5	0.0	0.0	0.0	0.0	0.0	0.0
14	R6	0.0	0.0	0.0	37.5	40.0	38.5
17	R6	0.0	0.0	0.0	0.0	0.0	0.0
21	R6	16.7	0.0	12.5	0.0	66.7	40.0
24	R6	0.0	16.6	8.3	50.0	66.7	55.5
28	R6	0.0	11.1	7.1	20.0	20.0	20.0
10/1	R6	0.0	18.7	15.0	62.5	60.0	61.1
5	R7	0.0	0.0	0.0	38.5	40.0	39.1
8	R7	0.0	20.0	13.6	30.0	33.3	31.8
12	R7	0.0	0.0	0.0	35.3	33.3	34.4
15	R8	0.0	0.0	0.0	50.0	25.0	40.0
19	R8	0.0	11.8	10.5	0.0	25.0	15.8
22	R8	0.0	0.0	0.0	0.0	0.0	0.0

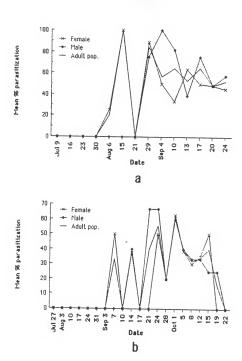


Figure 6. Mean percent parasitization of N. viridula adults collected in soybean. a) 1986, b) 1987.

In neither year, was it possible to determine a peak period of consistent levels of parasitization for  $\underline{N}$ .  $\underline{viridula}$  adults by  $\underline{T}$ .  $\underline{pennipes}$  in soybeans. Parasitization levels varied greatly from week to week. It is interesting to note, that in 1987, the mean percent parasitization sharply declined during the last two sample dates. In the last sample, October 22 (R8) no parasitized  $\underline{N}$ .  $\underline{viridula}$  adults were present.

Mean percent parasitization of N. viridula adults was examined for each soybean growth stage for each year. These values represent the means obtained during all samples for each growth stage (Table 17). In 1986, the value obtained in R3 growth stage was not considered because only one female was collected (and she was parasitized). In 1986, parasitization of adults occurred consistently during R5, R6 and R7 soybean growth stages, but consistency in parasitization in 1987 occurred during R5, R6, R7 and R8. In 1986 during R5, female and male levels of parasitization were the highest, and in 1987 this peak occurred during the R6 and R7 growth stages.

Although another species of egg parasite emerged from eggs of N. viridula on other crops, the only parasite species that emerged from soybean collected egg masses during 1986 and 1987 was <u>Trissolcus basalis</u>. During the 1986 soybean season, 137 egg masses were collected, totaling 8318 eggs, and 2793 were parasitized, giving an

Table 17. Mean percent parasitization of N. viridula adults by  $\underline{\text{T}}$ .  $\underline{\text{pennipes}}$  in soybean for each sampled growth stage, 1986 and 1987.

Growth stage	nª	Female	SEP	Male	SE	Total	SE
Year:	1986						
V5	1	none	-	none	-	none	-
V8	1	none	-	none	-	none	-
V10	1	none	-	none	-	none	-
R2	2	25.0	*	0.0	-	20.0	*
R3	1	100.0	*	none	-	100.0	*
R4	1	none	-	none	-	none	-
R5	1	88.9	-	75.0	-	84.6	-
R6	5	48.9	4.7	68.7	11.2	57.6	3.2
R7	1	45.4	-	57.4	-	52.5	-
Year:	1987						
V9	1	none	-	none	-	none	-
R1	1	none	-	none	-	none	-
R2	2	none	-	none	-	none	-
R3	1	none	-	none	-	none	-
R4	2	0.0	-	0.0	-	0.0	-
R5	2	25.0	25.0	0.0	-	16.7	16.7
R6	6	28.3	10.6	42.2	11.2	35.8	9.3
R7	3	34.6	2.5	35.5	2.2	35.1	2.1
R8	3	16.7	16.7	16.7	8.3	18.6	11.6

a) number of samplesb) standard errorb ased on one parasitized female

average egg parasitization level of 32.5%. Forty-one egg masses were parasitized, giving a mean egg mass parasitization level of 29.9%. Levels of parasitization varied from 0 to 65.8% for eggs and 0 to 62.5% for egg masses during July 25 to September 24 (Table 18). This was the period when vegetative and reproductive stages were important for stink bug feeding.

In 1987, from July 28 to October 13, 99 egg masses totaling 8562 eggs were collected and only 679 eggs were parasitized, giving an average egg parasitization level of 17.9% for the season. Twelve egg masses were parasitized, giving a seasonal average egg mass parasitization of 24.9%. Variation in the level of parasitization was very broad (0-100%) for both eggs and egg masses (Table 19). Combining both years data (Table 20), the average parasitization of N. viridula eggs by T. basalis in soybean was 24.9% and for egg masses was 22.0%.

Dr. F. D. Bennett raised the issue that time of sampling of an egg mass may have a direct influence on the result of its parasitization. This may be due to reduced time of natural exposure for potential parasitization.

This agrees with a previously mentioned source of bias (Chapter IV) where parasitization is prevented by removing the host or interrupting the parasitization process.

Table 18. Parasitism of N. viridula eggs and egg masses during the 1986 soybean season. N=number collected, P=number parasitized and %= percent parasitized.

		Egg ma	sses		Eggs			
Date	N	P	%	N	P	%		
7/25	5	0	0.0	_a	-	-		
29	3	0	0.0	-	-	-		
30	2	0	0.0	-	-	-		
8/01	2	1	50.0	-	-	-		
4	5	2	40.0	-	-	-		
8	2	0	0.0	-	-	-		
12	4	0	0.0	-	-	-		
15	9	1	11.1	-	-	-		
19	4	2	50.0	-	-	-		
22	2	0	0.0	-	-	-		
25	8	5	62.5	611	273	44.6		
29	6	6	37.5	1249	823	65.8		
9/03	7	3	42.8	493	141	28.6		
4	3	0	0.0	258	0	0.0		
8	9	1	11.1	648	78	12.0		
10	8	1	12.5	736	24	3.7		
15	15	6	40.0	1292	427	33.0		
19	18	6	33.3	1575	460	29.2		
22	7	3	42.8	646	161	24.9		
24	8	4	50.0	810	406	50.1		
Total	137	41	29.9	8318	2793	32.4		

a) egg number was not counted until August 22

Table 19. Parasitism of  $\underline{N}$ .  $\underline{viridula}$  eggs and egg masses during the 1987 soybean season. N=number collected, P=number parasitized, and %= percent parasitized.

		Egg ma	sses		Eggs				
Date	N	Р	%	N	Р	%			
7/28	2	0	0.0	183	0	0.0			
29	1	0	0.0	116	0	0.0			
30	4	0	0.0	398	0	0.0			
8/03	5	0	0.0	413	0	0.0			
5	2	0	0.0	154	0	0.0			
11	1	0	0.0	85	0	0.0			
13	2	0	0.0	129	0	0.0			
18	2	0	0.0	198	0	0.0			
25	1	0	0.0	66	0	0.0			
27	6	1	16.7	619	84	13.5			
31	1	0	0.0	80	0	0.0			
9/02	1	0	0.0	89	0	0.0			
3	1	0	0.0	78	0	0.0			
7	1	0	0.0	77	0	0.0			
8	2	1	50.0	189	88	46.6			
10	2	0	0.0	189	0	0.0			
15	5	1	20.0	309	30	9.7			
17	3	0	0.0	253	0	0.0			
19	4	0	0.0	323	0	0.0			
21	1	0	0.0	96	0	0.0			
22	10	0	0.0	848	0	0.0			

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Table 19 continued.

		Egg ma	sses	Eggs				
Date	N	P	%	N	P	%		
24	1	0	0.0	75	0	0.0		
25	8	1	12.5	560	2	34.1		
28	1	1	100.0	108	6	5.5		
29	6	1	16.7	569	2	34.0		
10/1	1	0	0.0	91	0	0.0		
2	7	2	28.6	646	140	21.7		
5	1	0	0.0	55	0	0.0		
6	9	1	11.1	912	1	0.1		
9	6	3	50.0	559	290	51.9		
13	2	0	0.0	154	0	0.0		
Total	99	12	12.1	8562	679	17.9		

Table 20. Summary of N. viridula egg and egg mass parasitization during the 1986 and 1987 soybean seasons. C=number collected, P=number parasitized and %-percent parasitized.

1986					1987			Both years		
Stage	С	P	%	C -	P	%	C	P	%	
E1	8318	2793	32.4	8562	679	17.9	16880	3472	24.	
EM <sup>2</sup>	137	41	29.9	99	11	11.1	236	52	22.	

<sup>1)</sup> E=egg

<sup>2)</sup> EM=egg mass

During 1986 and 1987, each egg mass collected was categorized into one of 5 previously mentioned categories. Two categories that I assumed resulted from avoidance and/or interruption of the parasitization process were yellow-fresh and yellow-old, but the remaining three were assumed to have had enough time for natural parasitization. Parasitization levels for yellow-fresh and yellow-old were practically the same, 22.6 and 27.7% respectively, but may suggest that yellow-old egg masses that were exposed longer in the field were more heavily parasitized (Table 21). Dark egg masses, had the highest level of parasitization but should be considered with reservation due to the fact that the dark color could be strongly related to its parasitized condition.

In 1986 and 1987, the most common species of fire ant collected on N. viridula egg masses was the red imported fire ant, Solenopsis invicta. This species represented 66.6% and 96.3% of the ants collected in 1986 and 1987, respectively.

Solenopsis geminata was also collected. In 1986, S. geminata was collected during the first egg exposure (July 7, V-8) but not during the 12 times egg masses were exposed in 1987.

Conomyrma burei was captured on two dates in 1986 (August 18 and September 1) and only once in 1987 (September 15).

Solenopsis invicta was the dominant species preying on southern green stink bug egg masses during the 1986 and 1987 soybean seasons. Stam et al. (1987) obtained the same results in their soybean field studies in Louisiana. The levels of predation in

Table 21. Percent parasitization of  $\underline{\mathrm{N}}$ .  $\underline{\mathrm{viridula}}$  egg masses for each category according to its condition when sampled, 1986 and 1987.

		Soybean				Other hosts		Total	
	1	986		1987					
Egg mass condition	No.	%	No	. %	No.	%	No.	%	
yellow-fresh	26	30.8	16	12.5	11	18.2	53	22.6	
yellow-old	53	30.2	12	8.3	18	33.3	83	27.7	
hatching	25	16.0	23	4.3	15	33.3	63	15.9	
dark	13	76.9	6	100.0	3	100.0	22	86.4	
hatched	18	29.4	42	7.1	6	0.0	66	7.6	

both years are presented in Tables 22 and 23. The level of predation (percent of egg masses taken) in 1986 was consistently much higher than in 1987. In both years, the largest amount of egg mass predation occurred during the first 24-48 h of exposure (91.4% in 1986 and 45.6% in 1987), It was very rare that egg masses were removed after 72-96 h of exposure. In 1986, the total number of exposed egg masses remaining after 96 h (4.3%) was drastically lower than those exposed in 1987 (45.8%). During 1986, 70 egg masses were exposed and 67 were removed, giving a 95.7% level of predation, and in 1987, 240 egg masses were exposed and 130 were taken, giving a predation level of 54.2%. Combining both years, the average rate of predation during 96 hours was 63.5% (310 egg masses exposed and 197 removed).

Table 22. Number of  $\underline{\rm N}.$  viridula egg masses (total of 10 per date) removed when exposed to predators in a soybean field in Alachua Co., 1986.

Date	Growth	No. of	egg	masses	remove	removed per hour		
	stage		24	48	72	96	Total removed	
7/07	V8		5	4	1	0	10	
29	V10		4	4	1	0	9	
8/08	R2		7	3	0	0	10	
11	R3		8	2	0	0	10	
18	R4		6	3	0	0	9	
25	R5		7	3	0	0	10	
9/01	R6		6	2	1	0	9	
Total			43	21	3	0	67	
Percen	t	61	. 4	30.0	4.3	-	95.7	

Table 23. Number of  $\underline{\text{N. viridula}}$  egg masses (total of 20 per date) removed when exposed to predators in a soybean field in Alachua Co., 1987.

Date	Growth	No. o	of egg	masses	remove	d per hour	Total
	stage		24	48	72	96	removed
7/2	V9		14	4	0	1	19
8/04	R1		16	2	1	0	19
10	R2		14	5	1	0	20
18	R2		14	3	0	0	17
25	R3		9	5	1	0	15
9/01	R4		0	0	0	1	1
8	R5		5	7	2	0	14
15	R6		6	3	3	0	12
22	R6		7	0	0	1	8
29	R6		1	1	0	0	2
10/6	R7		1	1	0	0	2
13	R7		0	1	0	0	1
Total			87	32	8	3	130
Percent	:		36.2	13.3	3.3	1.2	54.2

# CHAPTER VI EFFECT OF TRICHOPODA PENNIPES PARASITIZATION ON NEZARA VIRIDULA LONGEVITY, FECUNDITY, AND FEEDING IN SOYBEAN

## Introduction

The effectiveness of <u>Trichopoda pennipes</u> as a biocontrol agent against <u>Nezara viridula</u> is dubious. Some consider this parasite ineffective (Capeluto 1949) because it deposits more than one egg per individual host and the host continues to mate and oviposit despite being parasitized. Others believe it is effective (Harris and Todd 1982) because longevity of parasitized <u>N. viridula</u> is reduced causing significant reductions in population levels of this pest. These opinions were based on observations in particular situations such as a particular crop within a season. Any control tactic may succeed or fail or have a reduction in its potential for controlling an insect depending on several factors which vary from year to year.

In the literature, the range in seasonal average level of parasitization of N. viridula by T. pennipes is reported to be between 40-90%, but during a season parasitization levels can vary between 0-100%. Trichopoda pennipes is an active parasite present during almost all year in the southeastern United States. The key conditions for successful parasitism as stated by Bouletreau (1986) are based on the persistence of parasite-host interaction on

the parasites ensuring the dispersion of propagules to new hosts, and the acquisition of sufficient energy from the host to assure growth and survival of the host until reproductive maturity of the parasite. The presence of the parasite and host in the same habitat is the result of active behavior of the parasites (Bouletreau 1986).

Shahjahan (1968) studying the effect of <u>T</u>. <u>pennipes</u> parasitization on <u>N</u>. <u>viridula</u> reported reduced fecundity and longevity of parasitized compared with unparasitized bugs. Harris and Todd (1982) showed that parasitization by <u>T</u>. <u>pennipes</u> caused a 49% reduction in the longevity of male and female <u>N</u>. <u>viridula</u>. Egg fertility and egg mass size were not reduced by parasitization and fecundity of parasitized females was not reduced relative to that of unparasitized females during a time period equal to the lifetime of parasitized females. Lifetime fecundity of unparasitized females was 3.8 times the lifetime fecundity of parasitized females. They concluded that since <u>N</u>. <u>viridula</u> mate and oviposit throughout their lives parasitization by <u>T</u>. <u>pennipes</u> can cause significant reductions in population levels of this pest.

Besides the direct damage caused by the southern green stink bug to soybean seeds, it also causes indirect (secondary) damage to plants and consequently to seed quality and quantity. Secondary effects include delayed leaf maturation, retention of leaves and development of abnormal leaflets and pods close to the main stem (Todd and Herzog 1980, Panizzi and Slansky 1985). These secondary effects are normally present when extensive stink bug injury occurs during seed development.

The objectives of this study were to evaluate the direct effects of parasitization by  $\underline{T}$ .  $\underline{pennipes}$  on aspects of  $\underline{N}$ .  $\underline{viridula}$  longevity, fecundity and feeding in soybean.

## Materials and Methods

The experiment was conducted in 1987 on soybean variety Bradford, maturity group VII. A very uniform area in a soybean field on David Hodge's farm in Newberry, FL. was selected to conduct the experiment. A detailed field description is given in Chapter V. It was a caged experiment. Cages were similar, made of black plastic 0.5 mm mesh screen placed over a "tomato wire frame" and supported by wooden posts. The cone-shaped frame was placed up side down over the plants, i.e. the larger portion (opening) toward the soil surface. The cone was formed by 3 lateral wires, one of which was inserted in a hole in the stick and used as support for the cage. The two remaining wires were maximally extended to expand the overall diameter of the frame.

A rectangular screen bag (60 x 1.20 m) with Velcro the length of one side was placed over the frame and firmly adjusted around the frame. The screen cage was gathered and fastened with clothespins at the base of the plant

stems in such a way that there was no opening. A heavy layer of Tanglefoot TM (sticky material) was applied to the bottom of the cage and plant stems down to the soil surface. This material was replaced or reapplied every 2-3 days or immediately after a rain to prevent ants and other predators from entering the cages. Each cage enclosed 3-5 uniform plants. Cages were set up at the beginning of the R4 soybean growth stage to avoid possible injury to pods or seeds by stink bugs from the field population. The cages were placed in pairs 2 meters apart along rows. One row was skipped between rows with cages. Several plants adjacent to the cage were removed to prevent leaves from touching the screen and being used as a bridge by ants for entering the cages. Before the cages were set up, all spiders, insects and eggs were removed from the plants by shaking and hand-picking. Thereafter, regular checks were made to insure that no insects were in the cages before commencement of the experiment.

Parasitized adults were obtained by exposing individuals of the same age (7-10 days old) to fly parasites for a 24 h period, after which each bug was checked for presence of tachinid eggs on the body. Those with 2 or more eggs were removed and paired. No effort was made to pair the sexes by size or color. Only two exposures of host to parasites were necessary to obtain the needed number of parasitized couples. To replace dead or

injured bugs, the same procedure was conducted with a large number of adults maintained on pole bean pods at 27°C, 70-80% RH, 14 h photophase. Unparasitized pairs were obtained in the same manner with insects being taken from laboratory rearing cages. Nezara viridula nymphs were reared on pole bean pods and peanuts and adults maintained on pole bean pods Each pair, parasitized and unparasitized, was placed in a small container with a piece of soft tissue paper and immediately taken to the field and introduced into cages. This procedure avoided or minimized stress caused by higher temperatures, excessive handling or other physical injuries. At the beginning of R5 growth stage on September 9, one female and one male adult N. viridula were introduced into each cage. Nine cages received a parasitized pair and nine received an unparasitized pair. The paired cages (parasitized and unparasitized) were set up at the same time and the sequence of cage pair infestation was done at random (choice from 1-9).

If mortality occurred for any adult during the first 4 days after introduction into the cage the pair was removed (both dead and alive) and replaced by another pair from the laboratory stock of the same age and time of parasitization.

Each cage was observed daily during the morning hours (9-11). Three days a week (Monday, Wednesday, Friday) the

cages were opened on the Velcro side and inspected for egg masses and conditions of adults were observed. On other days, external observation through the screen was made to note conditions of adults. Dead stink bugs were removed from the cage on the day of discovery and brought to the laboratory to record parasitization. Egg masses also were removed when observed and the number of eggs per egg mass and egg fertility were determined in the laboratory. Egg masses were maintained in a plastic petri dishes (15 x 90 mm) with a water saturated dental cotton roll inside the petri dish to maintain high humidity and stored in a rearing chamber at 27°C, 70-80% RH and 14 h photophase. Each cage was checked until both insects died, and thereafter only maintenance was done (TanglefootTM maintained and dead leaves removed). The cages were removed and plants were cut off at the bottom of the stem on the day of harvest. All pods were picked by hand. In the laboratory number of filled and empty pods were counted. Seeds were removed from pods and number of seeds, weight and number of punctures per seed were recorded. These observations were made separately for each cage.

Soybean seeds were separated and classified into categories described by Jensen and Newson (1971). The four categories were based on the amount of visual damage from the southern green stink bug, such that N = seeds with no visual damage, L = seeds showing punctures but without

shriveling of the seedcoat, M = seeds with some shriveling of the seedcoat, H = seeds with extensive or total shriveling of the seedcoat. These seed categories were adopted and used. Seeds were also categorized according to the number of punctures per seed: 0, 1-3, 4-7, and more than 7 punctures per seed.

After all seeds of each cage were classified, using these two classification systems, they were vigorously mixed and passed through a seed laboratory sieve No. 9. They were separated into normal (not passed through the sieve) and abnormal (passed) seed. All seeds of each category were counted and weighed.

A microscope and an illuminated magnifier were used to search for punctures and determine the damage category. One person made all of the observations. For the purposes of this study, fecundity refers to the number of eggs laid, fertility is indicated by change in egg color from yellowish-white to orange-red or hatched, and infertility is indicated by eggs not changing color hue (except yellow) or unhatched after two weeks.

## Results and Discussion

This experiment was conducted over a period of 53 days, and 52 daily observations in cages were made. One cage with a parasitized pair was lost, due to a hole in the screen from which both insects escaped after 6 days.

Results reported were obtained from 9 cages with

unparasitized pairs and from 8 cages with parasitized pairs.

Raw data on longevity and fecundity obtained from both treatments are presented in Appendix B. Longevity, number of days lived, was significantly different for females, males and total days lived for the pair. Unparasitized insects lived much longer than parasitized (Table 24). Parasitized females lived a mean of 15.0 ± 2.7 days, with a range of 7-33 days, while unparasitized females lived a mean of 29.0 + 4.4 days with a range of 13-48 days. Parasitized males lived a mean of 12.0 + 2.4 days, with a range of 5-26 days, compared to unparasitized ones that lived 29.2  $\pm$  4.9 days with a broader range of 5-52 days. Longevity for total adults, the total number of days that both sexes in a pair lived, was much higher for unparasitized insects, with a mean of 59.0 ± 6.4 days (range 34-89 days). Parasitized adults lived a mean of  $27.0 \pm 4.9$  days (range of 13-59).

Parasitization of N. viridula adults by T. pennipes affects female and male longevity, reducing their life span by 44.9% and 36.4%, respectively. Number of cumulative days lived for each sex and for each combined pair was calculated. It was assumed that this combined value represented the amount of time that plants in each cage were potentially exposed for feeding. It also was assumed

Table 24. Effect of  $\underline{T}.$  <u>pennipes</u> parasitization on  $\underline{N}.$  <u>viridula</u> adult longevity.

		Longevity (Days)				
Condition		Mean	Range	SE		
Unparasitized	Female	29.0 **	13-48	4.4		
	Male	29.2 **	5-52	4.9		
	Total	58.2 **	18-100	6.4		
Parasitized	Female	15.0	7-33	2.7		
	Male	12.0	5-26	2.4		
	Total	27.0	13-59	4.9		

T-test (P=0.05) \*\* significant

that the higher the number of cumulative days lived, the greater the feeding potential.

Parasitized females, males and total adults had a mean number of cumulative days lived (feeding days) of 15.0, 12.0 and 27.0 days, respectively. These values for unparasitized were 29.7, 29.3 and 58.2 days, respectively. The number of feeding days for each pair was calculated by adding the number of days lived by the female and male in the same cage (cumulative number of days lived for the pair). Parasitized cages had a mean number of feeding days of 27.0 (range 13-59 days) and unparasitized had more than double the number of days, 59 with a range of 18-100 days.

Fecundity, expressed by the number of egg masses or eggs produced, was statistically different (P<0.05) between parasitized and unparasitized females. Unparasitized females laid more egg masses and eggs. Mean numbers of egg masses and eggs per unparasitized female were  $2.5 \pm 0.53$  and  $199.3 \pm 15.0$ , respectively, and for parasitized female these values were  $0.6 \pm 0.19$  egg mass and  $51.2 \pm 47.9$  eggs, giving a significant mean difference (P<0.05) of 1.9 egg masses and 148.1 eggs per female (Table 25). Egg fertility was not statistically different (P>0.05), but was higher for unparasitized females (mean egg fertility 78.2%) than parasitized (mean egg fertility 48.7%).

Considering these parameters to indicate fecundity (number of egg masses and eggs) and fertility (percent

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Table 25. Effect of  $\underline{T}$ .  $\underline{pennipes}$  parasitization on  $\underline{\underline{N}}$ .  $\underline{viridula}$  fecundity and fertility.

Condition	Mea	n	Range	SE	
		Num	ber egg masses		
Unparasitized	2.5	**	0-5	0.53	
Parasitized	0.6		0-1	0.19	
		Number eggs			
Unparasitized	199.3	**	0-458	15.0	
Parasitized	51.2		0-86	47.9	
		Perc	ent Fertility		
Unparasitized	78.2	NS	0-100	14.4	
Parasitized	48.7		0-100	16.7	
T toot (D=0 05)	**	101	- A NO A		

T-test (P=0.05), \*\* significant, NS not significant

viable eggs) it was possible to demonstrate a drastic effect of <u>T. pennipes</u> parasitization on <u>N. viridula</u> females. This effect could strongly influence population growth. Parasitization effects fecundity, reducing by 82.1% the number of egg masses and 77.1% the total number of eggs produced.

Feeding activity of N. viridula adults had a direct effect on seed quantity and quality, and some parameters were measured to express differences due to the effect of parasitiztion by T. pennipes on N. viridula adult feeding. Unparasitized adults lived much longer than parasitized ones, consequently they had more feeding days and higher feeding activity. This effected pod and seed quantity and quality. Appendix C contains raw data obtained for seed evaluation. There was no statistically significant difference (P>0.05) between mean number of pods filled in cages with parasitized and unparasitized pairs, but cages with parasitized bugs had larger numbers of filled pods (11.8%), suggesting that parasitized adults fed less during the early stages of seed formation. The number of seeds produced in cages with parasitized and unparasitized adults were not statistically different, but again parasitized cages had a higher mean number of seeds.

The mean number of unpunctured seeds and, probably as a direct consequence, number of normal seeds were significantly different. The largest differences were

observed between means for these two variables. Cages with parasitized adults had nearly twice the number of unpuctured seeds and 1.45 times more normal seeds. Cages with parasitized adults had 23.8% fewer seeds punctured (30.9% of seeds punctured by parasitized bugs and 52.5% by unparasitized). According to the usual seed analysis for seed laboratory evaluation (J. B. Franca Neto, personal communication)1, cages with parasitized N. viridula had 93.8% normal seeds, and 98.4% of seed weight was from normal seeds. In cages with unparasitized N. viridula. these values were 78.1% and 94.0%, respectively. Cages with parasitized adults had 7.4% of the seeds unacceptable for sale as seeds (1.6% of the total seed weight) and unparasitized had 18.4% (6% of total seed weight). Table 26 presents principal effects of feeding activity of parasitized and unparasitized N. viridula adults. Total seed weight was not statistically different (P>0.05) between means of treatments with parasitized and unparasitized individuals, but cages with parasitized adults had a narrower range of variability in seed weight. This difference might be important when extrapolated to a larger amount of seed, for example, to the amount produced in a hectare.

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Table 26. Effect of  $\underline{T},\;\underline{pennipes}$  parasitization on  $\underline{N},\;\underline{viridula}$  adult feeding.

	Condition					
	Unparasitized		Parasitized			
Variable	Mean	Range	Mean	Range		
Pods filled	86.7NS	54-104	102.1	74-137		
Seeds unpunctured	68.3**	13-122	122.4	41-216		
Seeds punctured	75.4NS	40-134	54.7	14-151		
Total seeds	143.8NS	93-194	177.1	128-239		
Normal seeds	112.3**	6-167	163.1	99-228		
Seed weight mg	127.4NS	62.3-166.1	141.8	130.2-166.		

T-test (P=0.05), \*\* significant, NS not significant

CHAPTER VII
ASPECTS OF TRICHOPODA PENNIPES OVIPOSITION AND ITS RELATION
TO PARASITIZATION OF NEZARA VIRIDULA ADULTS

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#### Introduction

Organisms evolve in response to their changing environment, and the interaction between an exploiter and a victim is viewed as a series of co-adjustments. There is a general tendency towards a steady state, where both species can survive (Price 1980). It is certainly not in the interest of the exploiter (parasite) to exterminate the host. The interspecific relationship between Trichopoda pennipes and Nezara viridula has been studied in various levels of detail (Hokkanen 1985, 1986, Todd and Lewis 1976, Shahjahan 1968).

Shahjahan (1968) stated, based on his study of the effects of superparasitization of the southern green stink bug by T. pennipes that supernumeray oviposition by the parasite on the host (4-8 parasite eggs/host) seems to be desirable because only about 51% of the parasite eggs produce larvae which actually penetrate the host. He concluded that successful penetration is affected by the location of the parasite eggs, since the larva fails to penetrate the host when eggs are deposited on the antennae, proboscis or wing membrane. Todd and Lewis (1976) studied natural field parasitism of adult N. viridula by T.

pennipes to evaluate incidence and ovipositional patterns on eight alternate host plants. Their data show that regardless of the host plant type, males had a higher percent of parasitization and a higher mean number of parasite eggs per bug than females. They believe that the growth habitat of various host plants had a marked effect on the overall percent parasitization of both male and female N. viridula. Similar results were obtained by McPherson et al. (1982) who found that significantly more N. viridula males had more parasite eggs on their integument and although means of 2.6 parasite eggs per male and 1.7 per female were observed, both sexes yielded a mean of 1.1 parasite larvae per host.

Correa (1984) studying egg incidence of the tachinid Eutrichopodopsis nitens on N. viridula, found this parasite species had a higher preference for ovipositing on the thoracic region, with the highest frequency of eggs on the prothorax (either ventrally or dorsally). An average of 3.5 and 2.9 tachinid eggs were found on male and female N. viridula, respectively.

Supernumeray oviposition by tachinid parasites on N. viridula was found to be common whenever it was studied. Shahjahan (1968) studied parasitism of the southern green stink bug by Trichopoda pennipes pillipes in Hawaii, and concluded that superparasitization was very common under Hawaiian conditions. He used the term superparasitization

to describe when female <u>T</u>. <u>p</u>. <u>pillipes</u> oviposit on a previously parasitized southern green stink bug and more than one larva is found in a host. Under normal field conditions he found a range of 1 to 8 tachinid eggs per host and the highest numbers recorded were 237 eggs on a field-collected bug and 257 eggs on a laboratory-parasitized specimen. He found up to 80 dead larvae inside one host. In each case he studied, even though many parasite larvae penetrated the host, all except one died. Michael (1981) mentioned that <u>T</u>. <u>pennipes</u> maggots were able to penetrate the bodies of bugs only if eggs were laid in suitable positions. However, he did not indicate which positions were suitable, but stated that only one maggot developed in each bug.

One possible indicator of the <u>T. pennipes</u> population abundance might be the number of their eggs found on <u>N. viridula</u> adults. It is assumed that a high egg number per host would be a result of a large population, since <u>T. pennipes</u> sex ratio is believed to be 1:1 (Todd and Lewis 1976, Mitchell and Mau 1971). Number of <u>T. pennipes</u> eggs on hosts would be correlated with levels of parasitization.

Harris and Todd (1981b), through the mere presence of a tachinid egg on the cuticle, estimated N. viridula parasitization and wrongly determined 16.8% parasitism for bugs with eggs and 16.6% for bugs without eggs.

The objectives of this study were 1) to verify the incidence and oviposition pattern of <u>T</u>. <u>pennipes</u> on adults of the southern green stink bug collected from several host plant communities in Alaucha Co. and to identify various parts of the host body where eggs, once deposited, may fail to penetrate the bug, 2) to test the hypothesis that the higher the number of tachinid eggs on a host, the higher the parasitization, and 3) to estimate field parasitization by the number of tachinid eggs present on <u>N</u>. <u>viridula</u> adults.

### Materials and Methods

Some of the  $\underline{N}$ .  $\underline{viridula}$  adults collected from host plant communities (Chapter IV) were randomly selected for this study. There was no special collection procedure, but adults were collected whenever possible during the two years of the study, 1986 and 1987. Collection of adults over a long time period assured a larger variation and was more representative for bugs observed through time.

In an attempt to locate area(s) on the host body surface that were more or less susceptible to penetration by the tachinid larva, distribution of tachinid eggs was studied. All tachinid eggs present on the body surface of each bug were mapped on a dorsal and ventral diagram of the adult. When only one or two eggs were present, they were removed to verify the presence of the larval penetration hole under the egg. Sex, total number of tachinid eggs,

and effective parasitization were recorded for each bug.

To determine effective parasitization the same criteria as described in Chapter III were used.

For analysis of the oviposition pattern, the  $\underline{N}$ .  $\underline{viridula}$  adult body surface was divided into 19 "regions" summarized as follows: 1) antennae, 2) rostrum, 3) eyes, 4) ventral surface of the head, 5) total dorsal surface of the head, 6) total head 7) prolegs, 8) mesolegs, 9) metalegs, 10) total ventral thoracic surface, 11) total dorsal thoracic surface, 12) total thorax eggs, 13) scuttelum, 14) corium, 15) wing membrane, 16) under corium or membrane, 17) total ventral abdominal surface, 18) total dorsal adominal surface, and 19) total abdomen.

To examine the feasibility of estimating <u>T</u>. <u>pennipes</u> parasitization in the field through the presence of its eggs on the host body, visual observations of <u>N</u>. <u>viridula</u> adults found in a soybean field were made in 1986, from August 4 to September 19. These insects were collected and the presence of tachinid eggs on the body surface and sex were recorded and the insects left in the same area in the field.

## Results and Discussion

Tachinid eggs on  $\underline{N}$ .  $\underline{viridula}$  adults were consistently observed during the entire study period, in both years. One hundred and fifty samples were taken from 22 different sites during 1986 and 1987, covering a period from March to

October, and in all samples of <u>N</u>. <u>viridula</u>, most adults had tachinid eggs. Table 27 summarizes the incidence of tachinid eggs on adults, including the maximum number of tachinid eggs found per female and male host at each site, mean number of eggs per adult and standard deviation of the mean number of tachinid eggs recorded.

In 21 of the 22 sites sampled, N. viridula males had higher mean numbers of tachinid eggs per individual than females and one site had equal means. This indicates that males have a higher incidence of tachinid eggs on their body surface and also a higher maximum number of eggs on their bodies. Also males had higher standard deviation values suggesting a broader variation in number of tachinid eggs on their bodies (Table 27).

As mentioned before and according to results presented in Chapter IV, males had consistently higher parasitization. Harris and Todd (1980) stated that the sex ratio of N. viridula in the field was essentially 1:1, but they called attention to the fact that different parasitization levels between sexes may change this figure throughout the year. This was observed during both years. Always, a higher number of females was collected, regardless of host plant and time of sampling.

In total, during 1986 and 1987, 3959 N. viridula adults were collected from host plant communities and 2466

Table 27. Summary of the incidence of tachinid eggs on  $\underline{\text{N}}$ .  $\underline{\text{viridula}}$  adults collected in 1986 and 1987.

	Total pop.	Females			Males		
Site		rangea	meana	SDa	range	mean	SD
1	184	20	5.1	4.1	43	7.6	7.1
2	15	3	2.5	0.6	5	3.5	2.1
3	98	11	3.5	2.9	10	6.1	2.4
4	70	19	5.1	5.2	24	5.2	4.8
5	91	8	3.2	2.0	23	8.1	6.1
6	91	8	2.1	1.4	11	3.0	3.0
7	58	8	2.1	1.6	12	1.9	2.5
8	77	11	4.3	4.1	14	4.2	3.4
9	231	15	2.7	2.0	32	7.0	4.5
10	280	12	2.7	1.8	25	4.1	3.4
11	725	12	1.6	0.9	13	2.5	1.8
12	590	12	2.8	1.7	35	4.4	3.8
13	141	15	2.1	1.5	35	9.8	6.9
14	114	13	3.8	2.2	23	9.0	5.7
15	71	14	2.9	2.1	29	13.3	8.4
16	158	6	2.2	1.2	5	1.4	6.6
17	203	9	2.4	1.8	3	27.4	6.4
18	141	13	3.3	2.7	21	6.7	5.4
19	55	9	3.2	3.4	7	3.1	1.8

Table 27 continued.

Site	Total pop.	Females			Males		
		rangea	meana	SDa	range	mean	SD
20	64	2	1.1	2.5	6	2.8	1.4
21	245	5	2.4	1.3	8	2.9	1.8
22	195	6	1.9	1.3	6	2.1	1.3

a) range, max. number of tachinid eggs/individual; mean and standard deviation of the evaluated population.

(62.2%) were females and 1493 (37.8%) were males. From this total number of N. viridula adults collected, 649 insects, 324 males (49.9%) and 325 females (50.1%), were used to describe tachinid egg distribution.

Tachinid eggs were found on virtually all N. viridula body surfaces examined (Table 28). The highest concentration of tachinid eggs was on the thorax, with 1596 eggs (51.6%), followed by the abdomen with 1208 eggs (39.1%) and the head with 288 eggs (9.7%). The largest number of tachinid eggs was laid on the ventral surface of the thorax, 1103 eggs, representing 69.1% of all eggs laid on the thorax. The odiferous orifices are located there and probably account for such tachinid egg concentration. Mitchell and Mau (1971) suggested that males produce a pheromone (or another substance) that is highly attractive to the female tachinid. Trichpoda pennipes is a parasite that locates hosts by long-range chemoreaction (Mitchell and Mau 1971). This statement is repeatedly found in the literature, however, no reference was found that clearly mentioned scent glands as the organs responsible for releasing a substance attractive to female T. pennipes, but these data may suggest such a possibility.

Only 83 eggs (2.7%) were found on the appendages. It is very interesting that 347 eggs were laid on the hemelytra, and 98 of these were under or between the

Table 28. Number and distribution of tachinid eggs on N. viridula body surface parts. Head, categories 1-6; thorax, categories 7-12; abdomen, categories 13-19.

Category	Total	Percent	Range	Mean	SD
1	2	7.0	1	0.00	0.06
2	4	1.4	1	0.00	0.08
3	70	24.3	2	0.11	0.35
4	39	13.5	2	0.06	0.26
5	173	60.1	5	0.27	0.59
6	288	9.7	-	-	-
7	37	2.3	2	0.06	0.24
8	22	1.4	2	0.03	0.20
9	18	1.1	2	0.03	0.18
10	1103	69.1	2	11.70	2.21
11	416	26.1	7	0.64	1.00
12	1596	51.6	-	-	-
13	107	8.8	11	0.16	0.60
14	175	14.5	5	0.27	0.61
15	74	6.1	2	0.10	0.33
16	98	8.1	5	0.15	0.45
17	398	32.9	11	0.61	0.99
18	33	29.5	12	0.55	0.98
19	1208	39.1	-	-	-

membranous parts of the wings. It seems almost impossible for a female tachinid to lay eggs in this particular location. Eggs were also found under the corium.

Among adults with only one or two eggs, 43 (6.6%) were not truly parasitized (35 had one egg and 8 had two eggs). No particular position indicated an overwhelmingly higher resistance to maggot penetration. If such resistance exists, the pronotum (especially the proepisternum, mesosternum and mesepisternum) seems to offer the best possibility. Evidence of this possible resistance was observed on 12 occasions when insects with egg(s) on these areas were found and entrance holes were not present and therefore bugs were not truly parasitized. The maggot is able to penetrate into the host body without making a hole in the cuticle. Evidence of this was seen several times when a tachinid egg was found hatched, the host was parasitized and yet no maggot entrance hole was observed. Membranes (between sclerites) are a potential area for maggot penetration. I found that the larva enters the host body cavity through the hollowed podites, either the femur or tibia. A considerable number of eggs were laid on the eyes (70 eggs, 24.3% of eggs laid on the head).

To examine a relationship between the number of tachinid eggs on the host body and level of parasitization, a simple regression was used on data collected from 6 host plant communities (cowpea, cowpea mix, corn, cabbage, wheat

and lupine). There were significant relationships (P<0.05) between number of tachinid eggs and host parasitization for some host plants, such as corn and wheat where significance occurred for both sexes. In the cowpea and lupine host plant communities, there were no significant differences between these parameters. In cowpea mix, the relationship was significant only for females but at a low level (R<sup>2</sup> 0.16). In cabbage the relationship was significant only for males (Table 29). Figure 9 shows regression equations and coefficient of determination (R<sup>2</sup> values) for crops and sex where the the R<sup>2</sup> was higher than 0.50.

The data presented by Harris and Todd (1981b) about field estimation of <u>T. pennipes</u> parasitization based on egg presence on the host body was examined during the 1986 soybean season. Data collected in Georgia can only be roughly compared to these data because in Georgia many more observations were made during several years.

A total of 325 N. viridula adults were observed, and 227 had tachinid eggs on their body surface, representing an average of 69.8% (75.3% males and 68.1% females). This estimated percentage of "parasitization" was compared with data obtained through dissection of collected adults during the same period; the latter estimate was 62.4% (55.1% males and 69.8% females). The difference is 7.4% between the two methods for determining parasitization.

Table 29. Coefficient of determination ( $R^2$ ) between number of tachinid eggs and mean percent parasitization for female and male  $\underline{N}$ .  $\underline{viridula}$  on several host plants.

Host	Female	Male		
cowpea mix	.16	NS*		
cowpea	NS	NS		
cabbage	NS	.52		
corn	.46	.46		
wheat	.52	.60		
lupine	NS	NS		

<sup>\*</sup> not significant at P=0.05

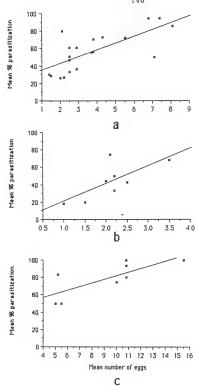


Figure 7. Correlation between number of tachinid eggs on host body and parasitization levels. a) cabbage, males; b) wheat, female; c) wheat, male.

This is not close to the 16.8% observed by Harris and Todd (1981b). However, it is important to determine a factor to correct such estimates. Obviously, this goal will be achieved only through an exhaustive research effort and is still to be done. Field estimation of N. viridula adult parasitization by the presence of tachinid eggs would be very valuable because it could be incorporated into the value compared to the threshold level of N. viridula in soybean. Currently, Florida threshold level is a mean of one and three bugs per 3 row ft, regardless of any parasitism or parasitization value.

# CHAPTER VIII

This was the first study conducted in Northcentral Florida with the purpose of quantitatively describing naturally occurring parasitism of the southern green stink bug, Nezara viridula, in the most common host plant communities during two consecutive years. Previous studies in Florida were conducted in selected crops during July, August and September (Drake 1920, Buschman and Whitcomb 1980). The present work was conducted in Alachua County, Florida, during 1986 and 1987.

The species of parasitic fauna found to be associated with N. viridula is believed to be representative. Levels of parasitization were highly variable and may have been influenced by drastic differences between summer weather during the two years. The first summer was rainy and the second was very dry.

Currently N. viridula rearing methods used by various researchers are basically the same. Some changes were undertaken in the present study to improve rearing efficiency. When nymphs were reared separately for each instar, mortality was drastically reduced, especially during IV and V instars. The small amount of extra work neccessary to seperate nymphs was rewarded by increasing

span and fecundity were increased and mold incidence on food sources was reduced.

Trichopoda pennipes is a tachinid species that can be reared under laboratory conditions. Data indicated that the Dietrick and Van de Bosh (1957) mass rearing technique for this species is improved by keeping containers with flies at a 45° incline under a continuous 15 watt fluorescent lamp and suppling a 20% honey water solution. New parasitization cages and containers should be used for each new quantity of host to be exposed to parasites. Newly mated females oviposit very intensively soon after being placed in a cage with a host. Oviposition declines after 24-48 hours. Under some environmental laboratory conditions (container, substrate, humidity, ventilation) T. pennipes puparia had an enormous inviability (did not emerge). The best condition found for holding puparia until adult emergence was to place puparia on the surface of a 1:5 moist vermiculite and washed sand mixture in a petri dish. Dishes were maintained in an incubator at 27°C, 70-80% RH and 14 h photophase. Daily, using an atomizer, a fine mist of tap water was sprayed on the puparia. Flies emerged predominantly during morning hours.

It was possible to identify the most common  $\underline{N}$ .  $\underline{viridula}$  egg opercula shapes produced by  $\underline{Trissolcus}$   $\underline{basalis}$  emergence. Data suggest the possibilty of using shapes of opercula to characterize parasitization when field

collected eggs have already hatched or parasites have emerged. T. basalis produced several shapes of opercular openings which were neither uniform or symetrical. If part of the operculum remains, it is a parasite emergence hole. Possibly, this technique could be adapted to estimate egg parasitism for other species if sufficient observations of parasite emergence could be observed. In my study only two parasitized egg masses by Opencyrtus submetallicus were collected, and for this reason it was impossible to characterize differences between species in terms of opercula shapes. I believe that a minimum of one hundred observations should be made to characterize a species. For T. basalis, 150 observations were made.

To evaluate parasitism, thirteen different crop species in 22 host plant communities were studied in Alachua Co. during 1986 and 1987. T. pennipes was the only parasite recovered from N. viridula adults collected from crop species in host plant communities on every sampling date. This species also was recovered rarely from IV and V instar nymphs. Hexacladia hilaris was recovered six times from N. viridula adults during the two year study.

Trichopoda pennipes was extremely predominant as the primary adult parasite of the southern green stink bug in the areas and years sampled. Levels of parasitization were different for each sex, and adult males had higher parasitization regardless of host crop or site sampled.

Trichopoda pennipes is active in early spring crops. Levels of parasitization increase during spring and summer months and decrease in late summer. During the two year period, an average of 54.9% of all N. viridula adults collected were parasitized by T. pennipes.

Among the host plant communities studied, cowpea mix showed the highest levels of  $\underline{N}$ . viridula parasitization and soybean the lowest levels for both females and males. In all other host plant communities, levels of parasitization were generally intermediate between levels in cowpea mix and soybean communities.

Both <u>T. basalis</u> and <u>O. submetallicus</u> parasites emerged from <u>N. viridula</u> eggs collected in host plant communities. <u>T. basalis</u> was by far the most abundant. Levels of parasitization by <u>T. basalis</u> were highly variable in all crops (0-100% for sample date and seasonal mean parasitization between 8.4-52.8%). Such variability is not characteristic of an ideal biological control agent, therefore there is a need for a more diverse egg parasite fauna exhibiting different behavior and field performance. Only two parasite species, <u>T. pennipes</u> and <u>H. hilaris</u>, attacked <u>N. viridula</u> adults and only <u>T. pennipes</u> attacked nymphs. Adult females were the first <u>N. viridula</u> stage collected in soybeans during both years. Nymphs were collected only during soybean reproductive growth stages. Adult female and male numbers of <u>N. viridula</u> were

practically equal in both years, as well as in their temporal distribution during the soybean season. Patterns of mean densities of N. viridula very similar in two years.

Nezara viridula egg mass and egg densities in the 1987 soybean field were extremely low until R6 when an average of 8 eggs/3 row feet were found. Nymphal populations in both 1986 and 1987 began increasing sharply in R6 and peaked in R7. In soybean the only N. viridula nymphal and adult parasite found during 1986 and 1987 seasons was T. pennipes. This species was present in soybean fields during the entire season when N. viridula was present. Nymphs had much lower levels of parasitization than adults. In neither year was it possible to determine a period of constant levels of parasitization for either adults or nymphs.

The only N. viridula egg parasite encountered in soybean was  $\underline{T}$ . basalis. Levels of parasitization were higher in 1986 than in 1987. In both years levels of parasitization by sample date were highly variable, 0-65% in 1986 and 0-51.9% in 1987. Egg masses collected from soybean fields showed that age had no effect on level of parasitization.

The red imported fire ant, <u>Solenopsis</u> <u>invicta</u>, was by far the most common predator of <u>N</u>. <u>viridula</u> egg masses in both soybean seasons. The majority of egg mass predation occurred during the first 48 hours of exposure. Seasonal

average predation level was 95.7% in 1986 and 54.2% in 1987 but the latter is still a high level for a single predator species, especially in an ecosystem where other fire ant prey are very abundant during portions of the season. A possible interaction between levels of egg predation by fire ants and egg parasitism may exist. While egg predation decreased during July-September, egg parasitism increased. This trend was clearly observed only during the 1987 soybean season and for this reason, I suggest that to confirm such a phenomenon would require further investigation.

<u>Trichopoda pennipes</u> parasitization had a strong negative effect on  $\underline{N}$ .  $\underline{viridula}$  adult longevity, fecundity and feeding. A parasitized female lived half the time of an unparasitized and for a parasitized male this reduction in longevity was even greater. Unparasitized females laid four times more eggs than parasitized. Parasitization had no significant effect on egg fertility.

Feeding intensity by N. viridula is reflected by number of puctured soybean seeds. Caged plants exposed to unparasitized adults produced only one half as many unpunctured seeds compared to cages with parasitized adults. The percentage of normal seeds (those with commercial value) was 31.1% higher in cages with parasitized adults. In order to study possible cage effects on soybean plants, further studies should be

conducted in which additional control cages are maintained with plants and no insects.

Eggs of T. pennipes were found on host bodies at all sample sites in both years. Supernumary ovipositon was found to be common on both male and female bugs. Males had a higher incidence of tachinid eggs than females and males had more eggs/individual. These facts may account for why males consistently had higher parasitization levels.

Examinination of tachinid egg distribution on major body parts of N. viridula adults revealed eggs were found virtually on the entire body surface. The highest concentration was on the thorax, followed by the abdomen and head. The largest number of eggs was laid on the ventral surface of the thorax. Since odiferous orifices are located in that region and T. pennipes locates a host by long-range chemoreaction, the large number of eggs observed may have been related to production of a pheromone or (substance) by male N. viridula which was highly attractive to the female tachinid. Very few eggs were laid on the appendages but considerable numbers were laid on the hemelytra, under or between the membranous part of the wings. No particular position of egg oviposited on adults was found that clearly indicated resistance to maggot penetration. If such resistance exists, the proepisternum, mesosternum and mesepisternum seemed to offer the best

possibilities because these parts of pronotum has the

A significant relationship between number of tachinid eggs on the host body and level of parasitization was found in the corn and wheat communities for both sexes. Data from the cowpea mix community showed a significant relationship for females, and in cabbage for males. Data from lupine and cowpea indicated no significant relationship between parasite egg number and level of parasitization.

The hypothesis that the more tachinid eggs per host was directly related to a higher propability of parasitization was found to be true for individuals with 3 eggs or less. When two tachinid eggs were present on a host only 0.957 were truly parasitized. With three eggs or more 0.99 were truly parasitized. I conclude that the number of tachinid eggs on the host does not improve chances for being parasitized with 3 or more eggs per host.

The results of my studies demonstrate that the principal factors involved in parasite-host relationships must be identified and quantified in order to evaluate the role that parasites play as natural agents for controlling a specific pest. Many factors influence the performance and efficiency of parasites. Quantitative estimates of parasitism indicated that levels were highly variable in all host plant communities studied and especially in

soybean. Such variability can result in adequate biological control of  $\underline{N}$ .  $\underline{viridula}$  in some years and not in others. A desirable goal for future studies would be to experiment with different approaches for decreasing the variability in adult stink bug parasitism by  $\underline{T}$ .  $\underline{pennipes}$ . Two basic approaches for increasing and providing more consistent levels of parasitism by  $\underline{T}$ .  $\underline{pennipes}$  should be considered: (1) conservation and augmentation of the soybean system and (2) importation and introduction of parasites.

The conservation of parasites seems to be especially important because this strategy may be implemented simply. quickly and without increasing production costs. One of the first goals in maximizing the use of biotic agents is the development and adoption of appropriate insect management practices that will conserve the natural agents already present and permit them to exert their potential regulatory effects. Improvements might be accomplished by careful monitoring of all pests and application of insecticides only when economic thresholds are exceeded. The cost of monitoring is usually offset by the savings from not spraying when unnecessary. Insecticides which are very damaging to natural enemies such as methyl parathion should not be used to control lepidopterous defoliators such as the velvetbean caterpillar and soybean looper when stink bugs are not present or are at low population levels.

such as the velvetbean caterpillar and soybean looper when stink bugs are not present or are at low population levels.

Augmentation or altering the environment so that established parasites and predators can function more effectively is another important strategy for increasing and stabilizing parasitism by T. pennipes. A possible technique would be the intercropping of soybean with other N. viridula host plants which do not produce large nymphal populations but maintain parasitized adults which that can move into soybean. In my studies, I observed that corn, cabbage and sunflower exhibit such properties. Before a crop for augmentation is selected, it is very important to study and determine if the possible benefits are greater than the negative effect of producing large quantities of pest inoculum for soybean fields. If corn, cabbage and sunflower do not produce harmful levels of N. viridula adults that could move into soybean, then they are good candidates for augmentative crops because these crops grow in Alachua Co. from May to early August, when levels of parasitization of N. viridula adults are low in soybeans. The presence of these crops in soybean producing areas may increase adult parasite populations, consequently providing more individuals for invading sovbean and attacking the first N. viridula colonizers.

A second approach for increasing and stabilizing parasitism of  $\underline{N}$ .  $\underline{viridula}$  adults is importation and introduction of parasites. Biotic agents from other areas, such as the tachinids  $\underline{E}$ .  $\underline{nitens}$  and  $\underline{T}$ .  $\underline{giacomelli}$  from Brazil and Argentina, respectively could be imported and established in Florida. Perhaps other areas that have not yet been explored for adult parasites of  $\underline{N}$ .  $\underline{viridula}$  such as Africa would produce good candidates for introduction into the United States.

I have discussed augmentation and importation of adult parasites as the favored approaches because effectiveness of egg parasites may be compromised by an antagonist, the imported fire ant, S. invicta. My studies indicated a possible interference in the effectiveness of T. basalis due to fire ant predation on N. viridula eggs. However, this antagonistic effect was observed only during the 1987 soybean season and may not be a widely occurring phenomenon. If predation by fire ants is not a frequent and widespread problem, importation of egg parasites of N. viridula should be considered. Several species of egg parasites are available in Asia and Australia, for example T. basalis biotypes from Australia and T. mitsukurii from Japan.

Although the approaches of augmentation and importation are strongly recommended as areas for future research, the benefits that could arise from these approaches would not be realized for a long time. However, there are results from my studies that could be beneficial in a few years if not immediately. The quantitative estimates of parasitism derived in my studies could be incorporated into existing static economic or action thresholds for treatment of stink bugs. Incidence of parasitism is not taken into account in present thresholds and should be considered in calculating action thresholds for insecticide application.

My study on feeding of unparasitized and parasitized N. viridula adults demonstrated that soybean plants fed on by parasitized stink bugs produced 31.1% more normal seeds than those fed on by unparasitized individuals. The currently used threshold is based on the density of IV and V instar nymphs and adult stink bugs and treats parasitized and unparasitized individuals with equal weight. Since few nymphs are parasitized during the season, parasitized nymphs can legitimately be ignored. However, adults should be separated into parasitized and unparasitized and each group given a different weight in the threshold computation. I suggest that the computation of a value to be compared to the current threshold value be as follows:

### T = IV + V + UA + W(PA),

where T is the value to be compared to the threshold, IV and V are the numbers of fourth and fifth instar nymphs and UA and PA are the numbers of unparasitized and parasitized adults respectively. The parameter W is the weighted value for parasitized adults and is a factor to represent the decrease in damage when soybean plants are fed upon by parasitized stink bugs. I estimated that unparasitized adult feeding results in 31.1% fewer normal seeds produced. Plants fed upon by parasitized adults will produce 31.1% more yield. Therefore, the value of W is (1 - 0.311) or 0.689.

The difference in the value to be compared with the threshold value is reduced due to the W weighting factor for parasitism. This difference could be important for populations near the threshold value and could possibly change a decision to spray. My 1986 season is a good example. Without considering parasitism, the value to be compared with the threshold at the R7 soybean growth stage (3.0 per 3 row feet) was 2.95. In this situation the value is close enough to the threshold value that an insecticide application would be recommended. Using the formula which incorporates the effects of adult parasitism the calculated value is 2.30 which is comfortably below the threshold.

The results from my study provide pertinent information about parasitism and predation of  $\underline{N}$ .  $\underline{viridula}$ . The

suggestion for conservation and augmentation should be incorporated into a  $\underline{N}$ .  $\underline{viridula}$  management program to enhance the effects of parasites. The findings of parasite effects on the economic threshold decisions may be used to reduced insecticide applications in soybean, thereby indirectly enhancing performance of parasites.

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 $\begin{array}{c} \text{APPENDIX A} \\ \text{PARASITIZATION OF } \underset{\text{NEZARA}}{\text{NEIDULA}} \text{ BY} \\ \underline{\text{TRICHOPODA}} \text{ } \underline{\text{PENNIPES}} \text{ IN HOST PLANT COMMUNITIES} \\ \end{array}$ 

Table Al. Parasitization and incidence of  $\underline{T}$ .  $\underline{pennipes}$  eggs on  $\underline{N}$ .  $\underline{viridula}$  adults collected from host plant communities in 1986 and 1987. Alachua Co. N=number collected, %=percent parasitized.

							T	achini	d Eggs	
	Fer	males	M	ales		otal Pop.	Fe	male	Ma	le
Date	N	%	N	%	N	%	rang	eª mean	range	mear
				Sample	site	No. 1b				
6/27	19	94.7	10	100.0	29	96.5	19	6.3	27	10.9
7/07	8	87.5	12	100.0	20	95.0	11	6.1	43	10.8
15	18	83.3	23	86.9	41	85.3	10	3.2	15	5.2
22	51	62.7	43	95.3	94	77.6	20	4.7	12	3.5
Total	96	75.0	88	94.3	184	84.2				
				Sample	site	No. 2				
7/07	7	28.6	3	66.7	10	40.0	3	2.5	5	3.5
19	3	0.0	2	50.0	5	20.0	0	0.0	1	1.0
Total	102	0.0	5	60.0	153	3.3				
				Sample	site 1	No. 3				
6/21	25	84.0	4	100.0	29	86.2	11	4 . 1	10	7.3
28	25	56.0	11	100.0	36	69.4	9	3.4	10	5.7
7/07	13	61.5	4	75.0	17	64.5	6	2.7	7	5.3
24	11	27.3	5	20.0	16	25.0	7	4.0	-	-
Total	74	62.2	24	79.2	98	66.3				
				Sample	site	No. 4				
7/07	14	78.6	6	66.7	20	75.0	15	3.4	24	6.2
19	11	45.5	6	33.3	17	41.2	19	7.2	4	3.5

							T	achinio	d Eggs	
	Fe	males	М	ales		otal Pop.	Fer	nale	Ma	le
Date	N	%	N	%	N	%	range	e <sup>a</sup> mean	range	mean
			Sampl	e Site	No. 4	contin	ued.			
8/07	18	50.0	15	80.0	33	63.6	13	4.8	15	5.8
Total	43	58.1	27	66.7	70	61.4				
				Sample	Site	No. 5				
7/14	5	100.0	4	100.0	9	100.0	7	3.4	23	10.7
22	21	90.5	6	83.3	27	88.9	8	3.1	12	7.6
8/07	26	76.9	9	66.7	35	74.3	6	3.2	17	6.1
20	18	72.2	2	50.0	20	70.0	8	3.2	-	-
Total	70	81.4	21	76.2	91	80.0				
				Sample	site	No. 6				
7/22	30	43.3	50	50.0	2	1.3	11	2.7	-	-
31	22	50.0	19	52.6	41	51.2	8	2.9	7	3.3
Total	42	54.8	49	46.9	91	50.5				
				Sample	site	No. 7				
6/17	13	38.4	17	64.7	30	53.3	3	1.6	7	2.5
25	16	50.0	12	100.0	28	71.4	8	2.6	12	3.7
Total	29	44.8	29	79.3	58	62.0				
				Sample	site	No. 8				
6/21	1	0.0	7	57.1	8	50.0	0	0.0	5	3.0
7/07	10	20.0	5	80.0	15	40.0	10	5.5	11	4.2

							Та	achinio	d Eggs	
	Fe	males	Ma	les		otal Pop.	Fer	nale	Ma	le
Date	N	%	N	%	N	%	range	eª mean	range	mean
			Sample	Site	No. 8	contin	ued.			
8/28	14	71.4	10	70.0	24	70.8	11	3.5	10	4.8
9/06	21	52.4	9	88.9	30	63.3	10	3.8	14	5.0
Total	46	50.0	31	74.2	77	59.7				
				Sampl	e site	No. 9				
6/12	19	31.5	41	51.2	60	45.0	3	1.6	5	2.4
17	33	45.4	27	59.2	60	51.6	6	1.7	5	2.3
23	24	79.1	34	97.0	58	89.6	15	4.1	25	7.6
30	18	88.9	13	92.3	31	90.3	7	3.3	32	16.7
7/08	13	53.8	9	22.2	22	40.9	10	3.2	10	6.0
Total	107	58.8	124	67.7	231	63.6				
			S	ample	site	No. 10				
6/12	33	63.6	18	88.9	51	72.5	6	2.5	6	2.7
17	25	76.0	22	95.4	47	85.1	7	3.2	12	4.9
24	19	78.9	8	100.0	27	85.1	7	2.7	25	9.1
30	2	100.0	1	0.0	3	66.7	1	1.0	-	-
7/07	9	55.5	2	50.0	11	54.5	7	4.5	-	-
10	5	40.0	5	60.0	10	50.0	1	1.0	1	1.0
14	17	58.8	10	100.0	27	74.0	8	2.8	16	4.4
23	29	62.0	38	76.3	67	70.1	12	3.5	19	4.5

							Ta	chinic	d Eggs	
	Fer	nales	Ma	les		otal Pop.	Feп	nale	Ma	le
Date	N	%	N	%	N	%	range	e <sup>a</sup> mean	range	mean
			Sample	Site	No. 10	) conti	nued.			
26	17	52.9	20	60.0	37	56.7	2	1.5	5	2.3
Total	156	64.7	124	80.6	280	71.7				
			S	ample	site 1	No. 11				
5/07	50	50.0	26	73.0	76	57.8	5	1.5	13	4.3
13	53	41.5	31	61.2	84	48.8	5	1.7	8	2.9
19	67	29.8	53	47.1	120	37.5	6	1.6	11	2.5
26	62	33.8	81	25.9	143	29.3	3	1.6	5	2.0
6/02	46	21.7	53	30.1	99	26.2	4	1.7	3	1.4
9	71	19.7	59	50.8	130	33.8	4	1.5	10	2.5
16	43	37.2	30	80.0	73	54.7	4	1.5	4	2.1
Total	392	32.6	333	46.2	725	38.8				
			S	ample	site 1	No. 12				
4/01	24	12.5	11	27.3	35	17.1	3	1.6	5	2.2
8	12	16.6	12	33.3	24	25.0	2	1.3	6	2.5
13	23	26.0	19	36.8	42	30.9	2	1.2	8	2.9
20	16	12.5	27	70.3	43	43.8	10	7.5	12	3.8
27	21	47.6	18	61.1	39	75.0	4	1.4	6	2.5
5/01	20	50.0	14	50.0	34	64.7	5	2.4	17	7.1
6	27	88.9	14	85.7	41	87.8	6	2.5	35	8.1
11	48	54.2	18	72.2	66	59.1	9	2.6	15	5.5

							T	achini	d Eggs	
	Fei	males	ľ	Males		Fotal Pop.	Fei	male	Ma	le
Date	N	%	N	%	N	%	rang	eª mean	range	mear
			S	ite No.	12 c	ontinued				
18	38	52.6	27	55.5	65	53.8	8	2.4	17	3.7
26	51	86.3	38	94.7	89	89.9	12	4.8	22	6.8
6/02	23	73.9	34	94.1	57	85.9	10	4.0	22	7.4
10	22	45.5	16	56.3	38	50.0	8	2.5	14	3.8
17	10	40.0	7	28.6	17	35.3	3	2.0	2	1.5
Total	335	53.1	255	68.6	590	59.8				
				Sample	site	No. 13				
4/02	11	18.2	4	50.0	15	26.7	1	1.0	7	5.5
8	5	20.0	2	50.0	7	28.6	2	1.5	-	-
15	9	44.4	5	80.0	14	57.1	4	2.0	25	10.8
22	9	33.3	6	100.0	15	60.0	4	2.2	35	15.5
27	8	50.0	8	100.0	16	75.0	5	2.2	23	10.8
5/01	22	68.2	15	93.3	37	78.4	15	3.5	24	10.8
6	20	75.0	6	83.3	26	76.9	11	2.1	12	5.2
11	7	42.8	4	75.0	11	54.5	4	2.5	20	10.0
Total	91	51.6	50	86.0	141	63.8				
				Sample	site	No. 14				
5/27	4	75.0	2	0.0	6	50.0	2	2.0	-	-
6/04	11	36.4	5	60.0	16	43.8	6	2.7	10	4.6
11	18	77.8	9	100.0	27	85.2	9	3.2	13	7.5

							T	achini	d Eggs	
	Fe	males	N	ſales	5	Total Pop.	Fei	nale	Ma	le
Date	N	%	N	%	N	%	range	eª mean	range	mean
			Si	te No.	14 c	ontinued				
19	4	100.0	3	66.7	7	85.7	7	4.6	23	16.5
23	21	90.5	5	80.0	26	88.5	13	5.3	17	7.6
7/02	4	100.0	0	0.0	4	100.0	11	7.7	-	-
9	5	100.0	1	100.0	6	100.0	5	2.5	-	-
16	13	69.2	3	66.7	16	68.7	9	3.4	-	-
23	5	80.0	1	100.0	6	83.3	5	3.0	-	-
Total	85	77.6	29	75.9	114	77.2				
				Sample	site	No. 15				
6/04	5	60.0	1	0.0	6	50.0	1	1.0	-	-
11	5	60.0	0	0.0	5	60.0	4	3.3	-	-
15	10	50.0	3	66.7	13	53.8	8	2.8	29	22.0
22	16	62.5	1	100.0	17	64.7	7	3.6	-	-
7/01	9	55.5	3	100.0	12	66.7	6	2.6	7	5.5
14	12	33.3	5	66.7	17	41.2	14	4.6	22	12.5
21	5	40.0	2	50.0	7	42.8	3	2.5	-	-
Total	62	51.6	15	66.7	77	54.5				
				Sample	site	No. 16				
5/20	56	14.3	39	33.3	95	22.1	2	1.2	5	1.7
25	20	25.0	10	10.0	25	20.0	4	2.2	-	-
30	18	27.8	8	12.5	26	23.1	6	2.8	1	1.0

							Т	achinio	d Eggs	
	Fe	males	Mε	ıles		otal Pop.	Fer	male	Ma	le
Date	N	%	N	%	N	%	range	eª mean	range	mean
			Sample	Site	No. 1	6 conti	nued.			
6/06	3	100.0	4	25.0	7	57.1	4	2.6	2	1.5
Total	97	21.6	61	26.2	153	24.2				
			5	Sample	site	No. 17				
5/14	4	0.0	4	25.0	8	12.5	-	-	-	-
20	12	33.3	11	18.2	23	26.1	3	2.0	-	-
25	8	37.5	4	75.0	12	50.0	2	1.2	5	3.3
30	9	22.2	8	87.5	17	52.9	4	1.7	17	8.6
6/07	9	11.1	13	69.2	22	45.4	1	1.0	16	5.1
17	2	100.0	4	75.0	6	83.3	1	1.0	11	6.7
30	18	100.0	11	90.9	29	96.5	5	2.8	22	5 . 4
7/07	15	80.0	5	80.0	20	80.0	29	5.5	28	12.7
14	12	75.0	8	100.0	20	85.0	7	3.5	32	11.3
22	19	52.6	8	87.5	27	63.0	6	2.2	7	4.1
30	9	88.9	10	90.0	19	89.5	8	3.5	41	9.3
Total	117	58.9	86	73.2	203	65.0				
			8	ample	site	No. 18				
6/02	2	100.0	5	100.0	7	100.0	7	4.5	21	10.0
10	16	62.5	4	50.0	20	60.0	13	3.7	19	12.0
17	18	61.1	4	75.0	22	63.6	11	3.7	5	2.6
26	7	28.6	5	60.0	12	41.7	7	3.3	13	6.3

							Та	achinio	d Eggs	
	Fer	nales	М	ales	7	Cotal Pop.	Fer	nale	Ma	le
Date	N	%	N	%	N	%	range	e <sup>a</sup> mean	range	mean
			Sampl	e Site	No. 1	8 conti	nued.			
7/01	10	70.0	0	0.0	10	70.0	5	2.5	-	-
6	6	33.3	1	100.0	7	42.8	7	3.5	-	-
14	10	70.0	7	57.1	17	64.7	8	3.5	12	8.0
21	17	82.3	7	57.1	24	75.0	6	2.9	4	2.7
28	14	64.3	8	87.5	22	72.7	5	2.6	13	5.5
Total	100	64.0	41	70.7	141	65.9				
				Sample	site	No. 19				
5/18	9	0.0	10	20.0	19	10.5	-	-	-	-
26	15	13.3	12	75.0	27	40.7	9	3.2	7	4.0
6/02	4	25.0	5	40.0	9	33.3	-	-	4	2.3
Total	28	10.7	27	48.1	55	29.1				
				Sample	site	No. 20				
3/09	13	23.0	9	66.6	22	40.9	2	1.2	4	2.3
13	5	0.0	7	57.1	12	33.3	-	-	6	3.2
17	4	25.0	8	50.0	12	41.7	-	-	6	3.0
23	6	33.3	7	57.1	13	46.1	1	1.0	3	2.7
4/01	4	25.0	1	0.0	5	20.0	-	-	-	-
Total	32	21.9	32	53.1	64	37.5				

							Ta	chini	d Eggs	
	Fe	males	M	lales	,	Total Pop.	Fem	ale	Ma	le
Date	N	%	N	%	N	%	range	a mean	range	mean
				Sample	site	No.21				
7/23	2	0.0	0	0.0	2	0.0	-	-	-	-
8/06	4	25.0	1	0.0	5	20.0	-	-	-	-
15	1	100.0	0	0.0	1	100.0	-	-	-	-
29	9	88.8	4	75.0	13	84.6	-	-	-	-
9/04	6	50.0	1	100.0	7	57.1	-	-	-	-
10	6	33.3	11	81.1	17	64.7	-	-	-	-
13	19	63.1	13	38.4	32	53.1	-	-	-	-
17	14	50.0	20	75.0	34	64.7	-	-	-	-
20	27	48.1	27	48.1	54	48.1	-	-	-	-
24	33	45.4	47	57.4	80	52.5	-	-	-	-
Total	121	50.4	124	59.5	245	54.5				
				Sample	site	No. 22				
7/27	1	0.0	0	0.0	1	0.0	-	-	-	-
9/03	2	0.0	1	0.0	3	0.0	-	-	-	-
07	2	50.0	1	0.0	3	33.3	-	-	-	-
10	6	0.0	1	0.0	7	0.0	-	-	-	-
14	8	37.5	5	40.0	13	38.5	-	-	-	-
17	2	0.0	1	0.0	3	0.0	-	-	-	-
21	2	0.0	3	66.7	5	40.0	-	-	-	-
24	6	50.0	3	66.7	9	55.5	_	_	_	_

							Tac	chinic	d Eggs	
	Fem	ales	Ma.	les		tal	Fema	ale	Ma.	le
Date	N	%	N	%	N	%	range	mean	range	mean
			Sample	Site	No. 22	conti	nued.			
28	5	20.0	5	20.0	10	20.0	-	-	-	-
10/1	8	62.5	10	60.0	18	61.1	-	-	-	-
5	13	38.5	10	40.0	23	39.1	-	-	-	-
8	10	30.0	12	33.3	22	31.8	-	-	-	-
12	17	35.3	12	33.3	29	34.4	-	-	-	-
15	18	50.0	12	25.0	30	40.0	-	-	-	-
19	7	0.0	12	25.0	19	15.8	-	-	-	-
22	8	0.0	1	0.0	9	0.0	-	-	-	-
Total	106	23.4	89	27.3	204	25.6				

a) Maximum number of eggs, minumum was always 0
 b) see Table 2 for site location, crop and period sampled.

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Table B1. Effect of parasitization by  $\underline{T}$ .  $\underline{pennipes}$  on longevity and fecundity of  $\underline{N}$ .  $\underline{viridula}$ .

Cage Sex lived  2 F 29     M 5 4 F 46     M 22 6 F 48     M 4 8 F 42	fed 34 68	Egg masses 2	Eggs 118 266	Cage	Sex F M	Day lived 71		Egg masses	Eggs 0
2 F 29 M 5 4 F 46 M 22 6 F 48 M 4	34	2 3	118	1	F	71		masses	
M 5 4 F 46 M 22 6 F 48 M 4	68	3					3	0	0
4 F 46 M 22 6 F 48 M 4			266	3	M	6			
M 22 6 F 48 M 4			266	3		0			
6 F 48	89			-	F	12	24	18	0
M 4	89				M	12			
		4	458	5	F	15	20	18	5
8 F 42					M	5			
	86	5	344	7	F	14	29	0	0
M 44					M	15			
10 F 13	65	1	68	9	F	15	21	1	75
M 52					M	6			
12 F 18	49	0	0	13	F	10	23	0	0
M 31					M	13			
14 F 25	51	4	256	15	F	33	59	1	86
M 26					M	26			
16 F 16	44	2	151	17	F	14	27	1	84
M 28					М	13			
18 F 30	45	2	133						
M 15									
Total F 267	531	23	1794		F	120	216	5	410
M 264					М	96			

APPENDIX C
SOYBEAN POD AND SEED EVALUATION

Table C1. Soybean seed evaluation of plants exposed to parasitized Nezara viridula by Trichopoda pennipes.

	Po	ods													
c a	f i 1	e m		eeds uncti	with ures	1		eds tego		r	No	rmal	Abı	normal	
g e	e d	p t y	0	1-3	4-7	+7	N	L	М	Н	N	Wt	N	Wt	Meana
1	103	18	41	106	35	10	41	78	64	6	183	24.8	9	. 2	.1302
3	115	9	145	53	1	0	145	17	20	17	182	28.6	17	. 4	.1457
5	137	18	216	23	0	0	210	13	6	10	228	33.6	11	.3	.1418
7	105	33	152	44	0	0	148	11	13	24	175	25.5	21	.6	.1331
9	95	29	119	41	0	0	118	20	16	6	155	26.4	5	. 2	.1662
13	111	16	109	54	2	0	107	37	3	18	148	28.9	17	.5	.1351
15	74	15	73	55	0	0	70	12	15	31	99	15.9	29	1.4	.1351
17	77	17	124	14	0	0	124	6	4	4	135	20.3	3	. 1	.1478

a) mean wt(g) of total seeds

Table C2. Soybean seed evaluation exposed to unparasitized  $\underline{\text{N. viridula}}$  by  $\underline{\text{T. pennipes.}}$ 

	Po	ods													
c a	f i 1	e m p	Seeds with punctures				Seeds per category				Normal		Abnormal		
g e	e d	y	0	1-3	4-7	+7	N	L	M	Н	N	Wt	N	Wt	Meana
2	102	10	50	122	12	0	46	77	21	40	150	24.4	34	1.2	.1391
4	89	78	73	45	0	0	53	10	25	30	104	18.9	14	. 7	.1661
6	54	44	12	61	18	2	2	13	29	49	26	3.2	67	2.6	.0623
8	79	36	55	57	3	0	42	15	26	32	92	14.3	23	1.1	.1339
10	69	70	3	43	47	26	3	20	41	55	46	6.2	73	2.8	.0756
12	104	24	122	47	0	0	116	32	9	12	159	24.6	10	. 3	.1473
14	101	24	87	107	0	0	69	59	31	35	167	25.3	27	.8	.1345
16	97	18	121	40	0	0	119	26	4	12	149	26.0	12	. 3	.1633
18	85	44	92	49	0	0	68	18	35	20	118	16.9	23	. 7	.1248

a) mean wt(g) of total seeds

## BIOGRAPHICAL SKETCH

Luiz Antonio B. Salles was born in Rosario do Sul, Rio Grande do Sul State, Brasil, on October 12, 1946. He received his B.S. degree in Agronomy in December 1970 from the Universidade Federal do Rio Grande do Sul. He received his M.S. degree in Entomology in November 1973 from the University of Sao Paulo/ESALQ.

He worked for Cia. Souza Cruz Ind. and Com. from January 1971 until June 1976, developing entomological research with tobacco pests. In July 1976, he was hired by the Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) to work on several biological control projects involving cereal aphids, apple mites, and sap beetles.

In January 1985, he enrolled in the graduate program of the University of Florida, Dept. of Entomology and Nematology.

Upon returning to Brasil, he will continue his work at the EMBRAPA as an entomologist specializing in biological control. I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr J. L. Stimac, Chairman Associate Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. D. C. Herzog Professor of Entomology and Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and  ${\cal C}$ was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy. Lack L. Fry

August 1988

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